Systematic Entomology

Systematic Entomology (2016), **41**, 717–731 DOI: 10.1111/syen.12183

Molecular phylogeny and generic-level taxonomy of the widespread palaeotropical '*Heteropsis* clade' (Nymphalidae: Satyrinae: Mycalesina)

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Abstract. The mycalesine butterfly genus *Heteropsis* Westwood, 1850 (Satyrinae: Mycalesina) has recently been conceived to be represented in three major palaeotropical regions (Madagascar, Africa and Asia), but there has been no formal taxonomic treatment covering this entire group. Studies aimed at understanding the evolutionary success of Mycalesina in the Old World tropics have been hampered by the lack of both a robust phylogeny and a stable nomenclature for this satyrine subtribe. Here, we present a well-supported molecular phylogeny based on 10 genes and 133 exemplar taxa, representing almost all known species groups of *Heteropsis* (s.l.), and including all but four known species in Madagascar. We also combine sequences of the exemplars with a morphological matrix of 428 characters. The widespread '*Heteropsis* clade' is confirmed as monophyletic, but lineages in different geographic regions also form endemic and well-supported clades with deep divergences among them. Here we establish this group as comprising three genera, *Heteropsis* (Malagasy region only), *Telinga* Moore, 1880 (Asia), and *Brakefieldia* **gen.n.** (Africa). We recover the genera *Telinga* and *Brakefieldia* as sisters with high support. Each genus is taxonomically characterized and a revised synonymic checklist is appended with new combinations and some changes in rank. With a well-resolved topology and updates to the taxonomy of the group, researchers are now in a position to explore the drivers of the spectacular radiation of the group, notably in Madagascar, where the highest phenotypic and species diversity occurs.

This published work has been registered in ZooBank, http://zoobank.org/urn:lsid:zoobank.org:pub:AAF9F440-A2D6-4483-BF35-9BC074D9D29B.

Introduction

The infusion of molecular data has undoubtedly revolutionized taxonomy. This invaluable toolkit has repeatedly highlighted shortcomings of traditional morphology-based taxonomy and systematics (Scotland *et al.*, 2003; Wiens, 2004). Morphological taxonomy has been found to be particularly inadequate in the case of rapid radiations characterized by high diversity of species and the paucity of species-specific diagnostic traits due to relative stasis in the evolution of observable morphological features (Kodandaramaiah *et al.,* 2010b; Van Bocxlaer & Hunt, 2013). The widespread occurrence of such species-rich taxa has made taxonomic investigations even more challenging, especially because of problems faced in accessing collections housed across several continents. Although butterflies are arguably the

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best-studied invertebrates, there are several species-rich groups whose taxonomy has not been satisfactorily resolved apparently due to the above-mentioned issues. One such group is *Heteropsis* Westwood, 1850, which has radiated successfully and rapidly across the three major biogeographic areas in the Old World tropics.

Heteropsis was first recognized from Madagascar as the leaf-mimicking species *Heteropsis drepana* Westwood. The affinities of this taxon were long obscure and intrigued naturalists (Wallace, 1876; Miller, 1968). As a result, *Heteropsis* has had a confused and complex taxonomic history, from a monobasic or dibasic genus (Mabille, [1887]; Ackery *et al.*, 1995) to one spanning Madagascar, Africa (Williams, 2014) and, more recently, Asia (Kodandaramaiah *et al.*, 2010a; Aduse-Poku *et al.*, 2015). The most recent of these last two treatments recognized this 'hairy-eyed' (i.e. compound eyes with dense intra-ommatidial setae) genus as one of the seven genera that constitute the satyrine subtribe, Mycalesina. This subtribe represents a spectacular butterfly radiation of over 300 species in the Old World tropics (Brakefield, 2010; Kodandaramaiah *et al.*, 2010a). Unlike the other mycalesine genera, which are regionally highly endemic, *Heteropsis* spans all major palaeotropical regions, with the largest diversification (∼74 species) occurring in the Malagasy region. The genus currently contains about 110 taxa occurring in both forested and open habitats in the Old World tropics, and some taxa have wing patterns that stand out from the rest of the subtribe.

The alpha taxonomy of the group in the Malagasy region was greatly clarified by Lees (1997), who synonymized 32% of the then known species and compensated this taxonomic loss with the discovery of about a third (23 undescribed taxa) of the currently known species on the island. In a follow-up work, Lees *et al.* (2003) proposed significant changes to the higher-level taxonomy for the Malagasy region by organizing 19 of the then recognized 45 species into five subgenera. The remaining 26 taxa were, however, considered *incertae sedis* (with unknown or undefined subgeneric groupings) in this treatment (Lees *et al.*, 2003). Since then, many more (∼25) new taxa of *Heteropsis* have been discovered in the region, and 19 of those are currently being described (e.g. Lees, 2016). Questions such as how the different species groups relate to one another phylogenetically and when the important divergences within the group occurred still remain, although Aduse-Poku *et al.* (2015) provided preliminary answers based on incomplete taxon sampling.

The African hairy-eyed mycalesine fauna has traditionally been placed in the genus *Henotesia* Butler (Gaede, 1931; Gabriel, 1932; Van Son, 1955; Usher, 1985; Kielland, 1994; Ackery *et al.*, 1995; Larsen, 2005; Libert, 2006). *Henotesia* was one of the five generic names downgraded to subgenus in Lees *et al.* (2003). The genus was introduced for the Malagasy *Henotesia wardii* Butler 1879 (representing a species now renamed as *Heteropsis viettei* Lees, 2003). In *Henotesia*, Oberthür (1916) had described four closely related Malagasy taxa (one still valid), but confusingly described corresponding females in the Asian genus *Culapa* (along with other species now known to represent endemic clades to the Malagasy Region). This trend of using *Culapa* was, however, followed by Turlin (1994). Aduse-Poku *et al.* (2015) showed that the genus *Culapa*, whose taxonomic scope these authors refined, has no direct relation with the Malagasy region Mycalesina.

Just as the use of *Henotesia* implies a link between Malagasy and African *Heteropsis* (s.l.), *Telinga* Moore, which was used as a subgenus in Lees *et al.* (2003) to include the Malagasy species *Heteropsis vola* (Ward), assumed a link with Asia. Following the molecular phylogeny of Aduse-Poku *et al.* (2015), these genus group names can, however, only be treated as endemic within discrete biogeographic regions. The apparently well-supported reciprocal monophylies of the '*Heteropsis*' lineages on the different continents (Aduse-Poku *et al.*, 2015) suggest that the grouping of *H. vola* and the Oriental taxa in *Telinga* is incorrect. Likewise the inclusion of the African taxa as part of the Malagasy genus *Henotesia* in some works (Gaede, 1931; Gabriel, 1932; Van Son, 1955; Usher, 1985; Kielland, 1994; Ackery *et al.*, 1995; Larsen, 2005; Libert, 2006) is deemed inaccurate. Furthermore, in Madagascar, the internal groupings in Aduse-Poku *et al.* (2015) for the group are not entirely in line with the preceding subgeneric classification proposed by Lees *et al.* (2003) in the Malagasy Region. In particular, the work of Aduse-Poku *et al.* (2015) shows that *Heteropsis fuliginosa* (Mabille), *H. drepana* and *H. paradoxa* (Mabille) were not placed with their closest relatives.

Considering groupings in the Asian clade, in addition to '*Mycalesis*' *adolphei* (Guérin-Ménéville) and '*Mycalesis*' *oculus* Marshall stated earlier (Lees, 1997; Lees *et al.*, 2003) to be part of the genus *Heteropsis* (as subgenus *Telinga*, now rendered polyphyletic), Kodandaramaiah *et al.* (2010a) recovered four additional Asian '*Mycalesis*' species [*M. sangaica* (Butler), *M. mamerta* (Stoll), *M. malsara* (Moore), and *M. janardana* (Moore)] as nesting within the otherwise Afro-Malagasy '*Heteropsis* clade' of their tree (see File S2 with regard to the correct application of the name '*mamerta*'). Aduse-Poku *et al.* (2015) recovered a further three *Mycalesis* taxa (*M. oculus*, *M. inopia* Fruhstorfer, and *M. misenus* de Nicéville) in the same clade of (Asian) *Heteropsis* (s.l.). These two recent studies (Kodandaramaiah *et al.*, 2010a; Aduse-Poku *et al.*, 2015) sampled about half of the (>100) Asian species previously placed in *Mycalesis*. In the absence of established morphological synapomorphies, increased molecular sampling of Asian *Mycalesis* (*s.l.*) is clearly needed. This would not only include the (>50) species that Kodandaramaiah *et al.* (2010a) established as *Mydosama*, but also those that Aduse-Poku *et al.* (2015) further separated out as 'true' *Mycalesis* or *Culapa.* Sampling of the African clade was also not comprehensive enough in Aduse-Poku *et al.* (2015) to test the species groups of Kielland (1984) in detail, except to confirm the deep division between the '*Heteropsis*' *peitho* group and the others.

As recounted in Aduse-Poku *et al.* (2015), most previous treatments of Mycalesina in general have been regional in scope. Only a couple of molecular studies (Kodandaramaiah *et al.*, 2010a; Aduse-Poku, 2015) have examined the group globally. Past phylogenetic studies of *Heteropsis* (Lees, 1997; Torres *et al.*, 2001; Linares *et al.*, 2009) have focused mainly on the taxa in the Afrotropical region, particularly Madagascar.

These studies clarified mainly the alpha-taxonomy of the group, leaving the higher-level taxonomy of the group largely unresolved and the evolutionary relationships of the different species groups unknown.

We here use a ten-gene dataset, with comprehensive sampling from Madagascar/Africa, as well as rich sampling from Asia and Australasia, to understand the phylogenetic relationships within the group, and also to resolve the higher-level taxonomy of the group. Based on the molecular evidence provided in this study, combined with morphological evidence for taxa unsampled for DNA, we provide for the first time a three-genus treatment of what we term hereafter the '*Heteropsis* clade' (= *Heteropsis s.l.* of the two most recent molecular studies). We compare and contrast the biogeography of this clade with the naked-eyed '*Bicyclus* clade' (*Bicyclus* Kirby and *Hallelesis* Condamin; Africa only) and the hairy-eyed '*Mycalesis* clade' (*Mycalesis* Hübner, *Culapa* Butler and *Mydosama* Moore; Indo-Australasia only) (see the phylogenetic hypothesis of Aduse-Poku *et al.*, 2015). A new genus is introduced for the African species within the '*Heteropsis* clade', while the other two genera are characterized morphologically and molecularly. The taxonomic composition and history of each clade is detailed, which we organize into species groups. We append a checklist of currently recognized species based on either molecules or morphology in this widespread palaeotropical clade with new combinations, including, where appropriate, revised subspecies statuses and synonyms that better reflect the morphological, genetic and biogeographic data. We consider it likely that the use of three genera for the '*Heteropsis* clade' will not only bring more impact in communication but will also prove more nomenclaturally stable in the future than the previous alternatives.

Materials and methods

Taxon sampling

Taxon selection was based on recently available taxonomic information on the *Heteropsis* (*s.l.*) groups in the different regions of the Old World tropics (Kodandaramaiah *et al.*, 2010a; Williams, 2014; Aduse-Poku, 2015). A total of 120 samples representing approximately 70 of the *c*. 74 so far known *Heteropsis* taxa in the Malagasy region were collected during our field expeditions between 2011 and 2014, or taken from the collections of D.C.L. and colleagues. This sampling includes all but two recently described species (Lees, 2016). Among the described and recognized valid species prior to Lees (2016), only the elusive and spectacular *Mylothris phileris* (Boisduval) mimic, *H. masoura* (Hewitson), for which the last confirmed museum collection is 1967, and the apparently narrowly endemic *H. anceps* (Oberthür) could not be sampled.

From mainland Africa, 14 exemplar taxa, representing 6 species of the African hairy-eyed mycalesines, were either collected in the field by K.A.P. or sampled from museum materials at the African Butterfly Research Institute (ABRI), Nairobi, and a few other sources. For the Asian radiation, we included 23 specimens from a set of 13 species from the Oriental group of *Heteropsis* (*s.l.*) as identified in previous studies (Kodandaramaiah *et al.*, 2010a; Aduse-Poku *et al.*, 2015). Outgroups included eight exemplars of *Bicyclus*, *Hallelesis*, *Mycalesis* and *Mydosama* species (all part of the Mycalesina subtribe) also following sampling in Aduse-Poku *et al.* (2015). Where possible, widespread ingroup species were represented by more than one sample, taken from different localities.

DNA isolation, amplification and sequencing

Genomic DNA was extracted from leg (and, in a few cases, thoracic) tissues of individual samples. DNA was amplified from ten gene regions; one mitochondrial [cytochrome *c* oxidase subunit I (COI)] and eight nuclear [carbamoylphosphate synthetase domain protein (CAD); Ribosomal Protein S5 (RpS5); Ribosomal Protein S2 (RpS2); wingless (wgl); cytosolic malate dehydrogenase (MDH); glyceraldehyde-3-phosphate dehydrogenase (GAPDH); elongation factor 1 alpha (EF-1α); isocitrate dehydrogenase (IDH); and Arginine Kinase (ArgKin)]. Primer-pairs for the polymerase chain reactions (PCRs) were taken from Wahlberg & Wheat (2008) and included the universal forward tail, which facilitated sequencing. All PCRs were performed in a 20 μL reaction volume. Successful amplicons were cleaned of single-stranded DNA and unused primers using EXO-SAPIT (Thermo Fisher Scientific/Affymetrix, Santa Clara, CA, U.S.A.). The cleaned amplicons were then sent to Macrogen Services in Amsterdam (the Netherlands) for nucleotide sequencing. Nucleotide sequence alignment was done by eye using BIOEDIT v. 7.2.0 (Hall, 1999). Sequences of different gene regions were then concatenated using VOSEQ (Peña & Malm, 2012). The software MEGA v6 (Tamura *et al.*, 2013) was used to assess the sequence properties of the individual genes and the multi-gene concatenated sequence matrix.

Phylogenetic inference

Phylogenetic inferences were made using two approaches; a maximum likelihood (ML) method and a Bayesian inference (BI) method. Optimal gene partitioning schemes and the best-fit model of nucleotide substitution for each partitioned dataset (at the codon level) were selected using PARTITIONFINDER (Lanfear *et al.*, 2012). This was necessary to minimize the effect of saturation and also to improve phylogenetic resolution of our multi-gene dataset. The eventual best model and partitioning scheme was decided based on a Bayesian information criterion (BIC; for the advantages of using this approach, see Sullivan & Joyce, 2005). To check for the degree of congruence among the different markers, phylogenetic analyses were first done separately for each gene (producing gene trees) and later for all ten genes combined, but partitioned by the optimal gene partitioning scheme suggested by the PARTITIONFINDER analysis.

Maximum likelihood analyses were implemented in RAXML-HPC2 v8.0.24, on the CIPRES Science Gateway v3.3 (Miller *et al.*, 2010), using the partition scheme from the PARTITIONFINDER analysis, under the GTRCAT model

for the rapid bootstrapping phase, and GTRGAMMA for the final best-scoring ML tree. For bootstrapping, we performed 1000 ML pseudo-replicate analyses. Bootstrapping was performed under the auto majority rule criterion (autoMRE). BI was carried out using Markov chain Monte Carlo (MCMC) randomisation in MRBAYES v3.2 (Ronquist & Huelsenbeck, 2003). We used reversible-jump MCMC to allow for sampling across the entire substitution rate models. Two parallel runs of four chains (three heated and one cold) were performed for 10 million generations, with sampling done at every 1000th generation. The software TRACER v1.6 (Rambaut *et al.*, 2014) was used both to estimate the sample sizes of the parameters in the BI and to check for the convergences or otherwise of the parallel MCMC runs.

Total evidence analysis

The total evidence approach as introduced by Kluge (1989) entailed utilizing all available relevant knowledge of the study system in a single analysis. Here, the analysis involved a combination of character data derived from morphology and the ten-gene DNA sequences used in the analyses described. We concatenated the 428-character morphological dataset of Lees (1997) with our molecular dataset for 139 exemplar taxa (including three outgroup taxa, *Bicyclus cottrelli*, *Hallelesis halyma* and *Mycalesis francisca*) (see Files S1 and S4). This analysis was aimed mainly at fitting the missing taxa in our molecular phylogenetic analysis (four in Madagascar and six in Africa) into a more comprehensive tree, but also allowed us to compare the datasets. For this analysis we pruned the aligned DNA sequences to retain only a single individual per species. The resulting matrix was chimeric and the dataset derived from morphology was added to this molecular dataset for as many species as possible, or otherwise scored as missing data. Phylogenetic analyses of the combined morphological and genetic (i.e. total evidence) dataset were performed using ML in GARLI v0.96 (Zwickl, 2006) and by BI using MRBAYES 3.2 (Ronquist & Huelsenbeck, 2003). Using the default parameters for the GARLI searches for mixed datasets, the analyses were run for 10 million generations. A total of 100 ML bootstrap replicates were also performed using GARLI and the resultant 100 trees were used to construct a majority-rule consensus tree with PAUP*4.0b10 (Swofford, 2003). The MRBAYES analyses were similar to those already described, except that the analysis was run for 20 million generations. To explore further where and how the addition of morphological data changed the topology or to appraise conflict or synergy between the datasets, we also generated a MRBAYES tree using only the above 428-character morphological dataset for comparison.

Taxonomic disclaimer

With the exception of *Brakefieldia*, **gen.n.,** any unpublished names in this work and its supplementaries are disclaimed for nomenclatural purposes (Art. 8.3 of the Zoological Code). However, nomenclatural acts implicit in all changed statuses, new combinations and synonymies herein, notably including those in Files S2 and S3, are not disclaimed.

Results

Dataset properties

The final dataset for the molecular phylogeny consisted of 165 exemplar taxa for about 97 species, of which 8 were outgroups. We were not successful in amplifying and subsequently obtaining sequences of all the ten target genes for all the exemplar taxa (see File S5 for a list of percentage gene coverage per taxon). The average gene coverage per exemplar taxon was *c.* 60%. The resultant combined sequence matrix contained 7735 aligned nucleotides, of which 36 and 25% were identified as variable and parsimony-informative sites, respectively. At individual gene levels, the mitochondrial gene COI had the highest number of variable and parsimony-informative sites, at 44 and 36%, respectively. As expected, the sequences of the nuclear genes were less variable, with, on average, 66% of their sites conserved.

Phylogenetic inference

The resultant phylogenetic trees from ML and BI analyses are largely congruent, the only difference between them being the degree of statistical support for the recovered nodes. In general, over 90% of the nodes in the proposed phylogenetic hypothesis are strongly supported with high bootstrap support (BS) values (BS > 75%; Fig. 1) and posterior probability (PP > 0.95; Fig. 2). All the '*Heteropsis* clade' taxa sampled formed a well-supported monophyletic group with strong support values (BS = 100, PP = 1). Likewise, the different *Heteropsis* (s.l.) lineages in the different geographic regions formed well-supported reciprocal monophyletic groups. The African clade (a new genus described in the following text as *Brakefieldia* **gen.n.**) and Asian clade (the genus *Telinga* stat. rev.), redefined here as including all newly combined Asian members of the 'Heteropsis clade' (Files S2, S3), are more closely related to one another than either is to the Malagasy region clade (genus *Heteropsis*, as circumscribed here) (Figs 1, 2).

Within the Malagasy region clade, there were three or four broad clades. One such broad clade is the *exocellata* species group, comprising *H. exocellata* (Mabille), *H. cowani* (Butler) and *H. mimetica* Lees & Kremen, in Lees, 2016, a rainforest, interior-restricted clade, which was found to be sister to the remainder of the *Heteropsis* radiation in Madagascar in the ML analysis (Fig. 1), albeit with weak support (BS = 49). Following this initial major divergence, the extant lineages of the group on the island were inferred to have diverged into two broad clades. One broad Malagasy subclade includes as its member taxa, the *H. narcissus* species group [*H. narcissus* (Fabricius) and *H. ankaratra* (Ward)], which comprise the most open-habitat or savannah-inhabiting species in the Malagasy region. Also included in this Malagasy subclade were taxa such *H. turbata* (Butler), *H. strigula* (Mabille), *H. sabas* (Oberthür), *H. andasibe* Lees and 19 others which have recently been

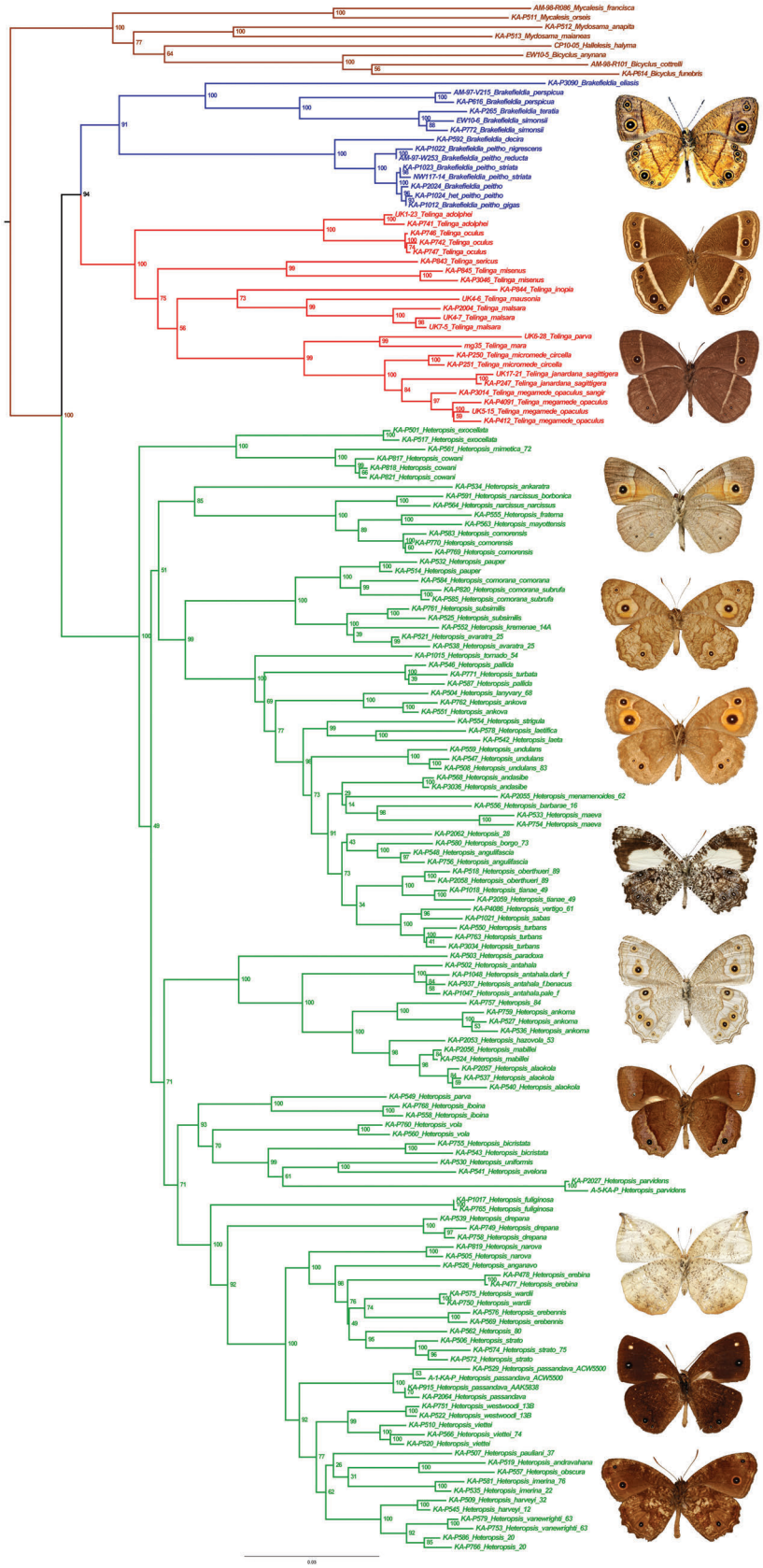

Fig. 1. Phylogenetic relationships of the different genera within the '*Heteropsis* clade' inferred from a maximum likelihood method. A (RAXML) maximum likelihood phylogeny reconstructed using the ten-gene concatenated dataset. The numbers at the nodes are the nodal support values from 1000 bootstrap runs. The colour-coded branches represent the outgroups (in brown) and the three genera (blue, *Brakefieldia*; red, *Telinga*; green, *Heteropsis*) within the '*Heteropsis* clade'. Representative images in ventral view, in descending order, are *Brakefieldia peitho* male (m); *Telinga mestra* – holotype female (f) of *Mycalesis mestra*; *T. sericus* m; *Heteropsis narcissus* m; *H. subsimilis* f; *H. turbata* m; *H. paradoxa* m; *H. antalaha* f; *H. bicristata* m; *H. drepana* f; *H. wardii* m; *H. obscura* m.

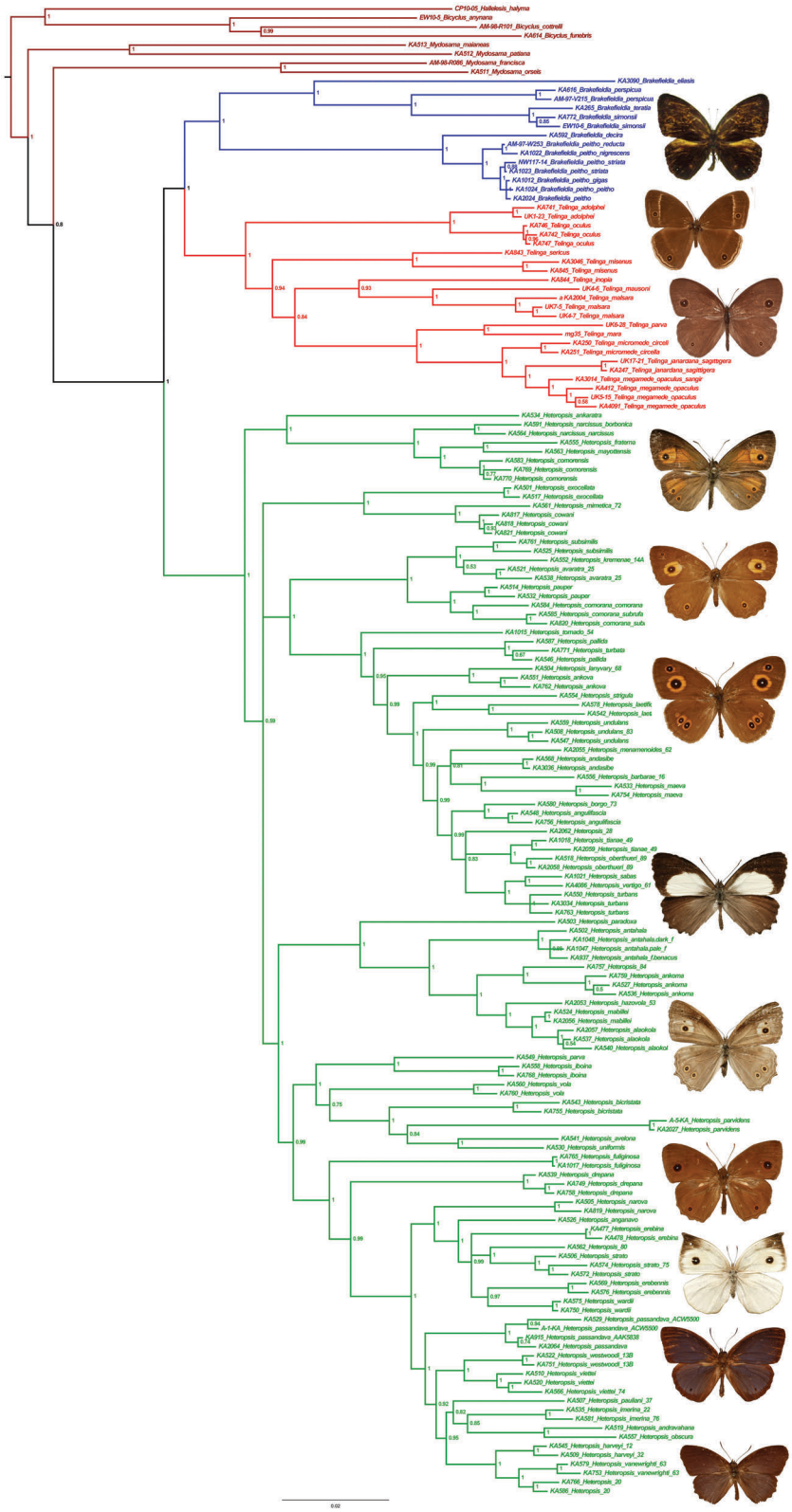

Fig. 2. Legend on next page.

described (Lees, 2016) or have being considered *incertae sedis* (unknown or undefined subgeneric groupings) by Lees *et al.* (2003). The other broad Malagasy subclade, by contrast, has the high-flying (primarily canopy specialists) in the region [such as *H. paradoxa*, *H. ankoma* (Mabille), *H. antahala* (Ward), *H. mabillei* (Butler) and *H. alaokola* (Oberthür)]. In addition to the high-flying species, this Malagasy subclade also contains the type species of the genus *Heteropsis* (*H. drepana*), the similarly intriguing *H. fuliginosa*, and taxa of the subgenus *Henotesia* as circumscribed by Lees *et al.*, 2003. Finally, it included as its member taxa, the enigmatic taxon *H. vola* (which was erroneously placed in *Telinga* in the earlier treatment) and members of the *H. iboina* and *H. avelona* groups (Fig. 1).

The BI topology, however, differed from the ML one in that the *H. narcissus* species group emerged as the sister clade to all other *Heteropsis* in the Malagasy region, a position assumed by the *H. exocellata* group in the ML analysis (Fig. 2), but this relationship was not supported (PP = 0.59). Aside from the *H. narcissus* group, the BI analysis recovered three clades within the Malagasy regional radiation, but the relationships between these broad clades were unresolved (Fig. 2). The *H. exocellata* group was one of these broad clades, with the other two clades being those recovered in the ML tree, but excluding taxa in the *H. narcissus* species group.

On continental Africa, there is a primary bifurcation in the African hairy-eyed lineages, leading to two long branches in both phylogenetic inference methods (Figs 1, 2). One of these two lineages is composed of *Brakefieldia peitho* (Plötz), **comb.n.** and five closely related taxa, which are strongly associated with dense rainforests in Central and Western Africa, but also occur in the Kakamega forest of East Africa. The other African clade comprises the open grassland and savannah adapted species groups (equivalent to the *ochracea*, *eliasis*, *elisi*, *perspicua* and *simonsii* groups of Kielland, 1994). These taxa are widely distributed in Eastern and Southern Africa, with *B. perspicua* (Trimen), **comb.n.** stretching to the highlands of Ethiopia. All African hairy-eyed mycalesines are newly combined here in *Brakefieldia* (File S2).

Within Asia, the endemic allopatric species pair *Telinga adolphei* and *Telinga oculus* from the Indian Western Ghats were recovered as sister taxa with strong support (BS = 100, PP = 1). Together these constitute the sister group of the rest of the Asian radiation in the '*Heteropsis* clade'. The remaining Asian *Heteropsis* taxa divide into two well supported clades with an almost perfectly allopatric geographic delineation. The taxa in the *T. sangaica* group and the *T. janardana* group (see Files S2 and S3 for the species list) are presently distributed in mainland Southeast Asia and on the islands of Southeast Asia, specifically in Malaysia, Philippines, Sulawesi and on some Maluku Islands. Taxa of the other well-supported clade within the Asian *Heteropsis* (s.l.) radiation [*T. inopia*, *T. malsara*, *T. mausonia* (Fruhstorfer), *T. misenus* and *T. sericus* (Leech), all new combinations] are, however, distributed mainly on tropical continental Asia with a distribution centred in and on the margins of the Indian subcontinent. File S3 provides all new combinations in *Telinga* and some revised statuses and is to be treated for nomenclatural purposes as an intrinsic part of this publication with the same date.

All the widespread species with multiple samples from different localities were recovered as monophyletic, confirming conspecificity. However, in some instances, the genetic divergences between two putative conspecifics were larger than 2% for the COI-5P region (Ratnasingham & Hebert, 2013). Similarly, the divergences between some putative subspecies (notably in *H. narcissus*) were relatively large in terms of genetic divergence.

Total evidence approach

The resulting matrix of the combined molecules and morphological characters contained 104 taxa and 8163 characters. All the ten molecularly unsampled taxa, represented in this combined matrix only by morphological data, grouped with their previously supposed morphological species groups (Fig. 3). The African taxa *B. angolensis* (Kielland), *B. centralis* (Aurivillius) and *B. phaea* (Karsch) grouped with the other three members of Kielland *perspicua-simonsii* group as a well-supported clade (BS = 90, PP = 1). The only members of Kielland's *elisi* group, *B. elisi* (Karsch), *B. ubenica* (Thurau), were recovered as sister taxa within the *Brakefieldia* radiation with high support (BS = 99, PP = 1). The remaining molecularly unsampled African taxon, *B. ochracea* (Lathy), was nested within the *Brakefieldia* radiation, with *B. eliasis* (Hewitson) as its sister taxon, but this relationship was weakly supported (BS = 50, PP = 0.94). The four molecularly unsampled Malagasy species, *H. masoura*, *H. anceps*, *H. sogai* Lees, 2016 and *H. roussettae* Lees & Kremen, in Lees, 2016, also grouped with their known species groups (Fig. 3).

However, compared with the molecular phylogenetic tree, the nodal support values were generally lower within species groups in the tree resulting from the combined morphology–DNA sequence matrix. At the deepest phylogenetic levels also, morphology did not perform well, in contrast to the phylogeny estimated using only molecules. For example, the tree estimated using only the 428 morphological characters failed to recover the '*Heteropsis* clade' as a monophyletic group. The three principal radiations (*Heteropsis*, *Brakefieldia* and *Telinga*) were recovered in the morphology-only phylogenetic analyses as either para- or polyphyletic (File S4).

Fig. 2. Phylogenetic relationships of the different genera within the '*Heteropsis* clade' inferred from a Bayesian method. A (MRBAYES) Bayesian phylogeny reconstructed using a ten-gene concatenated dataset. The numbers at the nodes are the posterior probabilities of 7500 trees. The colour-coded branches represent the outgroups (in brown) and the three genera (blue, *Brakefieldia*; red, *Telinga*; green, *Heteropsis*) within the '*Heteropsis* clade'. Representative images in dorsal view, in descending order, are *Brakefieldia peitho* male (m); *Telinga mestra* – holotype female (f) of *Mycalesis mestra*; *T. sericus* m; *Heteropsis narcissus* m; *H. subsimilis* f; *H. turbata* m; *H. paradoxa* m; *H. antalaha* f; *H. bicristata* m; *H. drepana* f; *H. passandava* m; *H. obscura* m.

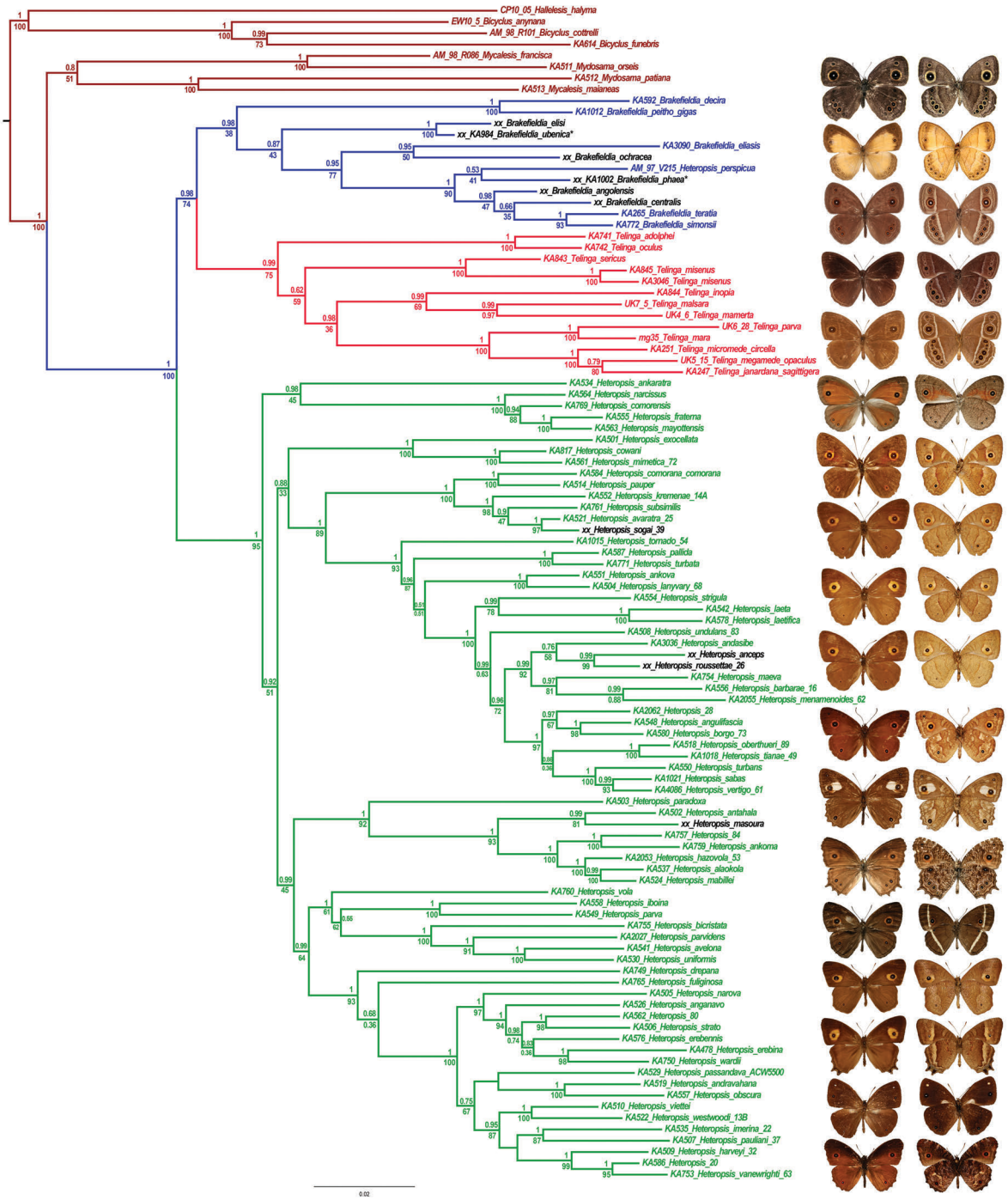

Fig. 3. Phylogenetic relationships of the different genera within the '*Heteropsis* clade' inferred using the combined dataset of morphological and ten-gene DNA nucleotide characters. A (MRBAYES) Bayesian phylogeny reconstructed using a combined data of morphological characters and ten-gene concatenated dataset. The numbers at the nodes are the posterior probabilities (above) and bootstrap support values (below) of the Bayesian (MRBAYES) and maximum likelihood (GARLI) methods, respectively. The colour-coded branches represent the outgroups (in brown) and the three genera (blue, *Brakefieldia*; red, *Telinga*; green, *Heteropsis*) within the '*Heteropsis* clade'. Exemplar taxa with names starting with 'xx_' and in black (e.g. *xx_Brakefieldia_elisi*) are taxa that were missed in the molecular phylogenies and here fit into the combined phylogeny using only morphological data. * denotes taxa that were considered rogue in the molecular phylogenetic analyses, because they only have a very short fragment of some genes. The embedded dorsal/ventral images, from the top down, are *Brakefieldia elisi* female (f); *B. eliasis* male (m); *B. perspicua* f; *Telinga sericus* m; *T. mara* f (paratype of *Mycalesis janardana mara*); *Heteropsis ankaratra* f; *H. comorana comorana* m; *H. pauper* m; *H. andasibe* m; *H. anceps* m; *H. angulifascia* m; *H. sabas* m; *H. mabillei* m; *H. vola* m; *H. parva* m; *H. avelona* m; *H. wardii* m; *H. viettei* m.

New taxonomy

Remarks. There are no available genus-group names that could be used for the taxa in Africa, hence the need for a new genus.

***Brakefieldia* gen.n. Aduse-Poku, Lees & Wahlberg**

Type species. Mycalesis peitho Plötz, 1880
http://zoobank.org/urn:lsid:zoobank.org:act:98C95114-EE13-4B7D-A492-2E12DB8E4433

Synonymy. None. Formerly known, incorrectly, as *Henotesia*.

Diagnosis. 596th and 598th nucleotides of the COI-5P barcode C and T (T and A in other two genera; first and third positions of a silent codon). No morphological synapomorphy has yet been identified. Kielland (1994) can be interpreted as suggesting not only that the base of R1 arises very close to the base of Rs1 along R but that the base of Rs4 at the cell ('base of vein 7': see Miller, 1970: Fig. 3) to the fork of R1 (vein 10) with R is about one-fifth of the length of the base of R to the base of Rs4 at the cell, but it is not currently clear that this parameter is significantly different from the other two genera in the '*Heteropsis* clade'.

Description. Kielland (1994: 236–237) gives a brief description of wings and male and female genitalia. Usher (1985) gives details of a few species. *Brakefieldia* are moderate-sized nymphalids, forewing lengths generally in range 18–24 mm (male) and 21–26.5 mm (female) (Kielland, 1994). Ground colours are predominantly light brown, yellowish, ochreous, orange, reddish or dark brown, wing shape never falcate, tailed nor crenate but hindwing margin gently rounded; forewings relatively broad and rounded in savannah species compared with both *Heteropsis* and *B. peitho* clade, which have somewhat narrower forewings (Kielland, 1994). Androconia is usually of the simple type with discocellular brush and patch, but there is a cubital brush in the deep forest *B. peitho* group (Usher, 1985: 259) juxtaposed with anteriad abdominal ventral black androconial scales (Lees, 1997: 98–99). In its sister, the 'savannah' clade, there are usually profuse leaden grey scales towards the base of the humeral area of the hindwing as far as spaces 1A or 2A (character 297 of Lees, 1997) not observed elsewhere in the '*Heteropsis* clade'. Ventral forewing androconia absent, inflated male hindwing veins absent except in anal system of *B. peitho* group. Male genitalia variable in shape, of the '*Heteropsis* clade' type, from lateral view with strongly waist at junction between tegumen and vinculum and valve with prominent shoulder close to base of gnathos, valve bases elongate and usually with narrow to sinuate valve arms, often proud of uncus tip except in *B. elisi* and *B. ubenica* where the valve tip is inflated, and in the *B. peitho* group where the valves are short and stubby (see Lees, 1997, pp. 101–102). There is an articulating process between the tegumen and valve base in the *B. peitho* group (Usher, 1985, p. 260). In most of the African 'savannah clade', the male genitalia exhibit a relatively stretched out shape, as, for example, also in the Malagasy *H. fraterna* (Butler), whereas *Heteropsis* in Madagascar are much more diverse in their configuration (Lees, 1997: 101–109). Uncus straight, to variably scythe-hooked, as common for the '*Heteropsis* clade'. Usually the valve tip ends on a small spine, but in *B. ochracea* there is a medial projection with spinoid setae. Aedeagus is usually recurved distad of ostium, nearly as long as the valve, and often featuring small spines towards the tip. Saccus is bulbous and never particularly long.

Etymology. In honour of Paul Brakefield, who has done a great deal to promote serious research of the subtribe Mycalesina over the last three decades.

The taxonomic history and reorganization of taxa in *Brakefieldia* are documented in File S2.

Discussion

Effect of improved sampling

The groupings are generally congruent among different analyses and with prior publications, particularly Aduse-Poku *et al.* (2015). Nodal supports are higher overall due to our relatively comprehensively sampled and more robust dataset and this is much more apparent compared with the preceding molecular treatments (Torres *et al.*, 2001; Kodandaramaiah *et al.*, 2010a). For instance, within the Madagascar radiation, almost all known taxa of *Heteropsis* in the region (see Taxon sampling, earlier) were included in this study.

Our molecular sampling is weaker outside Madagascar but we included at least 50% of recognized species in Africa and Asia. A little over 70% of our exemplars have at least five of the ten target genes amplified and sequenced, and overall coverage is improved more than three-fold (56 vs 157) in the number of ingroup exemplars since Aduse-Poku *et al.* (2015). In terms of species, the current study increased the number of '*Heteropsis*' taxa by 73% (56 vs 97) compared with Aduse-Poku *et al.* (2015). A number of the groupings and sister-taxon relationships recovered in our study were consistent with morphological similarities or synapomorphies in androconial and especially male genitalic structures that are less prone to homoplasy than, for example, wing pattern characters (Lees, 1997). Our phylogenetic estimate largely corroborates the general framework proposed by Aduse-Poku *et al.* (2015) for the group in their broader mycalesine study.

Systematic implications

Our results are consistent with the earlier concepts of *Heteropsis* (*s.l.*) as occurring in all the major palaeotropical regions (Lees, 1997; Kodandaramaiah *et al.*, 2010a; Aduse-Poku *et al.*, 2015). However, we have established for the first time and with strong statistical support the phylogenetic relationships of the three main and deep clades occurring on different continents of the Old World tropics. We retrieved the African and Asian lineages as sister clades which together shared a common ancestor with the Malagasy lineage before their divergence, as in

Aduse-Poku *et al.* (2015). Contrary to the diphyly origin hypothesis suggested in Kodandaramaiah *et al.* (2010a) for the Malagasy lineages, we found support for a single common ancestor for the Malagasy *Heteropsis* radiation in this study. Similarly, our results provide strong evidence for the monophyly of the African lineages, as opposed to the minimum of two independent radiations postulated by some earlier authors (Lees, 1997; Torres *et al.*, 2001). We also hypothesize that the Asian *Heteropsis* radiation also started off with a single common ancestor, just as in Africa and Madagascar. The biogeographic origin scenarios for these three continental clades of the '*Heteropsis* clade' are discussed extensively elsewhere (Aduse-Poku, 2015).

In the following, we discuss the implications of our results for the current classification and systematics of the '*Heteropsis* clade' in Madagascar, continental Africa and Asia. A disadvantage of the existing broad taxonomic system for *Heteropsis* as most recently circumscribed in Aduse-Poku *et al.* (2015) is that it does not formally recognize the evolutionarily deep clades in each region. Furthermore, the existence and use of subgenera in Lees *et al.* (2003) for the *Heteropsis* taxa in the Malagasy region mean that genus-group names (if available) for the groups in the Afrotropics and Asia can only be used at the subgeneric level. This potentially creates an imbalance in the treatment between regions. One possible proposition for addressing the aforementioned imbalance is to reorganize the extant *Heteropsis* taxa into three subgenera, with each of these subgenera representing each of the three monophyletic radiations in discrete landmasses or island groups. However, the splits between these clades are very deep, 18–26 Myr, and comparable to the estimated times of divergence between the mycalesine genera *Bicyclus* and *Hallelesis* in Africa, and between *Mycalesis* and *Mydosama* in Asia (Aduse-Poku *et al.*, 2015). More importantly, the genus *Heteropsis*, as until now recently circumscribed (equivalent to the '*Heteropsis* clade'), is in fact difficult to characterize morphologically and, at present, no reliable morphological synapomorphy has been recognized uniting all members, although the clade is well supported in the present and previous molecular studies (Kodandaramaiah *et al.*, 2010a; Aduse-Poku *et al.*, 2015). To acknowledge the deep divergences between the different regional *Heteropsis* clades and also provide a more stable taxonomic framework for the group, we provide here a three-genera treatment of the '*Heteropsis* clade' (= *Heteropsis* s.l. of the two most recent molecular studies). Under this treatment, each continental clade is given generic status.

Malagasy region

The genus *Heteropsis* was originally founded on the extraordinary sexually dimorphic leaf-mimicking taxon from Madagascar, *H. drepana*. We hereby restrict the use of *Heteropsis* to the hairy-eyed mycalesine radiation in the Malagasy Region. With this treatment the existing subgeneric names created in Lees *et al.* (2003) still remain useful, but as already pointed out in already previous studies (Kodandaramaiah *et al.*, 2010a; Aduse-Poku *et al.*, 2015) and also in this study, some of the subgenera do not constitute natural groupings. As at least an interim measure, here we use informal species group names instead of subgenera.

Compared with the *Heteropsis* fauna in Africa particularly, the taxonomy and systematics of Malagasy *Heteropsis* has only recently been the focus of comprehensive revisions (Lees, 1997; Torres *et al.*, 2001; Lees *et al.*, 2003; Linares *et al.*, 2009). In the latest revision of the group in the Malagasy region, Lees *et al.* (2003) subsumed the Malagasy genera *Houlbertia* Oberthür, 1916, *Admiratio* Hemming, 1964, *Masoura* Hemming, 1964, the Oriental genus *Telinga* Moore, 1880 and the Afro-Malagasy genus *Henotesia* into a single genus *Heteropsis.* Except for *Houlbertia*, which was completely sunk, all the subsumed genera were downgraded to subgeneric status. However, a little more than half (26) of the then 45 recognized species were considered *incertae sedis* (with unknown or undefined subgeneric groupings) in the circumscription (Lees *et al.*, 2003).

However, as already indicated, our proposed phylogenetic framework disagrees with some of the taxonomic treatments. Perhaps most notable among these disagreements is the grouping of Malagasy and Oriental taxa in *Telinga*, and Malagasy and African taxa in *Henotesia* in Lees *et al.* (2003). Taxa of the two canopy-occurring species groups (*Admiratio* and *Masoura*) formed a well-supported monophyletic clade. Taxa of these clades are also morphologically similar in their androconial structures. We therefore find it convenient on both molecular and morphological grounds to combine these two high-flying subgenera into a single group, the *H. antahala* group. More details of species and subspecies groupings of the Malagasy taxa are provided in File S2 and S3. Consistent with the phylogeny presented in this study, we have organised the ∼74 *Heteropsis* taxa in the Malagasy region into seven groups as the *H. exocellata*, *H. narcissus*, *H. subsimilis*, *H. strigula*, *H. antahala*, *H. iboina* and *H. drepana* species groups.

Our results regarding subspeciation provide a new framework to revise Lees *et al.* (2003)'s treatment of five to six related taxa as subspecies of *H. narcissus*. These taxa were originally introduced as separate species on the different islands in the Indian Ocean. Judging from the degree of genetic divergence between the different putative subspecies of *H. narcissus*, which falls well above the frequently regarded ∼2% maximum level in the standard COI-5P marker (DNA barcode) that characterizes intraspecific variation for about 95% of recognized animal species (Ratnasingham & Hebert, 2013), we recommend the reinstatement of four valid species [*H. narcissus* (Fabricius, 1798), **stat. rev.**, *H. mayottensis* (Oberthür, 1916), **stat. rev.**, *H. comorensis* (Oberthür, 1916), **stat. rev.** and *H. fraterna* (Butler, 1868), **stat. rev.**]. Here, therefore, purely on morphological grounds, we tentatively follow Turlin (1994) in splitting *H. comorensis* into two subspecies, ssp. *salimi*, which only occurs on Grande Comoro, and the nominotypical one, as occuring on Anjouan and (supposedly also) Mohéli. The type locality of '*Culapa*' *comorensis* in the original description (Oberthür, 1916) specifies only 'îles Comores par M. Humblot'; within a syntypic series of 14 specimens. However, Turlin (1994) designated (apparently validly: ICZN Code, Art. 74.5) a male specimen now labelled as lectotype of *C. comorensis* which he attributed to the Anjouan

population. Future work should further clarify the divergence between island populations of *H. comorensis*, as only Grande Comoro exemplars were sequenced in this study.

Mainland Africa

The African hairy-eyed mycalesine fauna has traditionally been placed in the genus *Henotesia*, based on the Malagasy *H. wardii* Butler (now *H. viettei* Lees, 2003). The above treatment therefore implies a close link between some Malagasy *Heteropsis* and the hairy-eyed taxa in Africa. This circumscription, as has been discussed earlier, is erroneous. The hairy-eyed taxa in Africa are even more closely related to the Asian ones than either is to the *Heteropsis* in the Malagasy region. In fact, Lees (1997) showed that the African hairy-eyed mycalesines should be excluded from *Henotesia*, for complete lack of morphological synapomorphies and cladistic groupings. Indeed, no morphological basis was established by earlier authors (e.g. Aurivillius, 1899; Gaede, 1931; Gabriel, 1932), who started or maintained the trend of placing the African hairy-eyed mycalesine species in *Henotesia*. Despite this, some authors (D'Abrera, 1997; Larsen, 2005; Libert, 2006) still retained *Henotesia* as the generic name for at least the bulk of the mainland African taxa in their taxonomic revisions.

Clearly from our results and also in the most recent molecular study of the subtribe Mycalesina (Aduse-Poku *et al.*, 2015), the sampled African hairy-eyed mycalesine taxa form a well-supported clade with no special or immediate relationship with Malagasy taxa. Consequently, we have erected a separate genus , *Brakefieldia*, for all the *Heteropsis* (*s.l.*) taxa occurring in Africa. Our study reveals two distinct clades within *Brakefieldia*. This division reflects both morphology and geographic distribution of the extant lineages of the group in Africa (Kielland, 1994; Larsen, 2005; Libert, 2006). One clade, comprising *B. peitho* and related species or subspecies, is known to be strongly associated with forest habitats in Western and Central Africa (Larsen, 1991, 2005; Kielland, 1994; Libert, 2006). The other distinct group of *Brakefieldia* is made up of ∼10 species distributed largely in grasslands and open habitats in the East and Southern Africa. Only one species (*B. elisi*) of this essentially savannah grouping reaches West Africa, prompting some authors (Usher, 1985; Larsen, 2005) to describe it as a biogeographical anomaly. Species of this clade are morphologically similar, especially in adult wing patterns and androconial structures; species delineations had been only possible in most cases by the use of genitalia (Kielland, 1990). We only managed to molecularly sample with adequate coverage 40% of these savannah and open-habitat *Brakefieldia* taxa in the present study. Many of these taxa are rare or occur in largely inaccessible countries as regards fresh material (Kielland, 1990).

However, integrating morphological characters with genetic sequence data for 135 Afro-Malagasy exemplars, all the six unsampled taxa grouped with their previously supposed morphological species groups (Kielland, 1994; Lees, 1997; Lees *et al.,* 2003). *B. angolensis*, *B. centralis* and *B. phaea* grouped with the other two members of Kielland's (1994) *simonsii* group (*B. simonsii* (Butler), *B. teratia* (Karsch), together with *B. perspicua*, which Kielland placed in its own group), as a well-supported clade in the combined matrix tree Fig. 3. *B. elisi* and *B. ubenica* were also recovered as sister taxa with high support. These two taxa share synapomorphic male genitalic characters (Lees, 1997) and were put together by Kielland (1994) as the only members of *elisi* group. We include as part of *Brakefieldia* the six molecularly unsampled savannah-adapted African hairy-eyed taxa revised in Kielland (1994), pending future molecular studies.

Taxa of the *B. peitho* clade are morphologically distinct from the savannah-adapted species, to the extent that D'Abrera (1997) even classified the former as belonging to Oberthür's Malagasy genus *Houlbertia*, but this treatment was refuted by Lees *et al.* (2003) and other taxonomic works (e.g. Larsen, 2005; Libert, 2006). The alpha-taxonomy of this rather small African clade has been in a state of flux in the recent past. Usher (1985) synonymized both *B. decira* (Plötz) and *B. nigrescens* (Bethune-Baker), arguing that they were but variants of *B. peitho* in the west and east of the Dahomey Gap, respectively. Libert (2006), however, disagreed with this assertion, also based on adult morphology (ocellus expression and wing morphometrics), and split the *B. peitho* complex into three species and six taxa with clear geographic delineations.

In this study, we have included exemplar taxa of all species of the *B. peitho* complex from Ghana, western Cameroon, Uganda, Kenya and southern Cameroon, Congo, equivalent to Libert's (2006) circumscriptions of *B. decira*, *B. peitho peitho*, *B. p. reducta* (Libert), *B. p. gigas* (Libert), *B. nigrescens nigrescens* and *B. n. striata* (Libert), respectively. On average, we found a relatively large (6%) genetic divergence between *B. decira*, occurring westward of the Dahomey Gap, and the rest of the *B. peitho* group taxa, occurring eastward of it. There is, however, very little difference (averaging less than 1%) in the (entire) mitochondrial COI (1475 bp) sequences between the three *peitho* group taxa (*B. p. peitho*, *B. p. reducta* and *B. nigrescens striata*) occurring eastward of the Dahomey Gap. Our results largely support Usher's (1985) position on this species complex, although not as a single species with two subspecies on either side of the Dahomey Gap as Usher had asserted, but rather as two allopatric species potentially separated by this gap. The same biogeographic division is implicated to have separated the only two extant species of the African endemic genus *Hallelesis* (Larsen, 2005). Based on the molecular evidence provided in this study and the morphological evidence presented in Usher (1985), we suggest that the names *B. decira* and *B. peitho* be reserved for populations of the *B. peitho* species complex occurring to the west and east of the Dahomey gap, respectively [a Ghanaian, Volta River, neotype for *Mycalesis decira* was designated by Libert (2006), for the former species].

Morphological synapomorphies have not yet been identified for the newly erected genus *Brakefieldia* (Lees, 1997). Kielland (1994) did not refer to any actual synapomorphies for this African hairy-eyed group. However, a consistent morphometric trait for the group is the very smooth noncrenate margin of the wings and the relatively broad wing shape for the taxa in the savannah clade. Taxa in the *B. peitho* species group, by contrast,

have narrower wing shape and anomalous male genitalia, which have relatively short and simple valves (Lees, 1997). No morphological character has yet been identified unifying all taxa of the savannah and forest *Brakefieldia* clades at the moment, so we base this subgenus on molecular evidence.

Kielland (1994) divided the African hairy-eyed mycalesine taxa into six species groups and we consider this taxonomic organization overly complex given their modest morphological differences and the phylogenies presented in this study. Three of Kielland's groups (the *B. peitho*, *B. perspicua* and *B. simonsii* species groups) have representatives in our molecule-only phylogeny (Figs 1, 2) and members of the remaining three species groups (the *B. elisi*, *B. ochracea* and *B. eliasis* groups) were included in the combined phylogeny (Fig. 3). To provide a stable taxonomic framework and to reflect the phylogenies presented in this study, we recognize two major groups within *Brakefieldia*, one restricted to dense rainforest (the *B. peitho* subclade) and one restricted to savannah and montane grasslands (the *B. eliasis* subclade).

Indo-Malesian region

Like their sister group in Africa, we consider that, on the basis of strong molecular evidence for the monophyly of our exemplars, along with some general morphological features, the Asian *Heteropsis* taxa should be assigned their own separate genus. Lees *et al.* (2003) adopted one of Moore's (1880) Asian mycalesine genera (*Telinga*) as a subgenus of *Heteropsis* in their classification, which included a Malagasy taxon, *H. vola*, and the Indian allopatric species pair, *Mycalesis adolphei* and *M. oculus*. This circumscription, as was discussed earlier, is erroneous, and rather, Malagasy and Asian radiations are locally monophyletic. We revise the genus Telinga to include all Asian members of the 'Heteropsis clade'. *Telinga* was originally described as monobasic, with *M. adolphei* as the only extant member. Only recently, the similar *H. oculus* was considered to be the allospecies of *M. adolphei*, separated by the Palghat Gap in the Western Ghats (Sekar & Karanth, 2013).

Until recently, *Heteropsis* (*s.l.*) was never suggested to occur in Asia. Because members of this group had never been separated from *Mycalesis*, the number of *Heteropsis* taxa in the Asian region is not yet firmly established, contrary to the case in Africa and Madagascar where about 12 and 74 described species, respectively, occur. In their study of the entire mycalesine radiation in the Old World tropics, Kodandaramaiah *et al.* (2010a) and Aduse-Poku *et al.* (2015) found eight Asian taxa, including the first suspected duo (*M. adolphei* and *M. oculus*) as belonging to 'Afro-Asian-Malagasy' '*Heteropsis* clade', as recently circumscribed. In the present study, we retrieved in this clade all of the '*Mycalesis*' recently placed in the '*Heteropsis* clade', together with an additional four taxa now placed in *Telinga*.

Our results reveal three well-supported clades within the *Telinga* radiation, reflecting both broad morphology and the present distribution of the extant taxa. These subclades are referred to informally here as the *Telinga*, *Samanta*, and *Martanda* subclades. Detailed descriptions of these subclades and their respective species groups are provided in File S2. Taxa of the *Telinga* subclades are remarkably distinct from those in the *Samanta* and *Martanda* subclades and incidentally share a few morphological characters (e.g. male genitalic and hindwing dorsal wing ocellus configurations) with the Afro-Malagasy taxa, prompting some authors in the past (Lees, 1997; Lees *et al.*, 2003) to associate them with the Malagasy taxon, *H. vola*, as their closest sister species.

This Indian-Malagasy association (based on possible morphological affinity) could be interpreted either as a convergence or as a retention of shared symplesiomorphic traits of the common ancestors of *Heteropsis*. This, then, would suggest that the Indian subcontinent served as the epicentre of the Asian *Heteropsis* radiation, considering also their local diversity. This hypothesis seems further strengthened by the recovery of the duo (*T. oculus* and *T. adolphei*) as sister to the rest of the radiation in the region; however, we did not recover *H. vola* as sister to the rest of the Malagasy radiation. Evans (1932) and Aoki *et al.* (1982) erroneously associated these taxa with *Mycalesis* (*s.s.*).

Leaving aside the primary split within the '*Telinga* subclade', the geographic disjunction between the *Samanta* and *Martanda* subclades seems to parallel that between the two main mycalesine genera in the region, *Mycalesis* and *Mydosama*. Taxa of these sister genera are nearly mutually allopatric, with *Mycalesis* taxa occurring predominantly in India, Indo-China and marginally into Sundaland, but taxa of *Mydosama* distributed within the Southeast Asian Islands in Sundaland, the Philippines, and far south and eastwards to the Solomons (Kodandaramaiah *et al.*, 2010a; Aduse-Poku *et al.*, 2015). In a similar pattern of geographic separation, species of the *Martanda* subclade occur mainly on the Southeast Asian islands (Sundaland, the Philippines and the Moluccas) and the southeastern part of mainland Asia, with *T. sangaica* extending its range to Japan. Taxa of the *Samanta* subclade are not known from any of the Southeast Asian islands. Instead they are distributed predominantly on continental Asia, with a strong presence on the Indian subcontinent (where no species from the *Martanda* subclade occurs). Without exception, all the seven sampled taxa of this subclade belong to one informal subgeneric unit called species group 3 in the latest classification of the group by Aoki *et al.* (1982). It therefore seems likely that most of the unsampled members of Aoki *et al.*'s (1982) species group 3 [*Mycalesis heri* Moore*, M. mestra* (Hewitson), *M. suaveolens* Wood-Mason & de Nicéville, *M. misenus* and *M. lepcha* (Moore)] might belong to *Telinga*, based on morphology. Indeed, apart from *M. suaveolens* (*incertae sedis*), all the others are newly combined with *Telinga* here (Files S2 and S3), based on molecular data where available and/or morphology.

Synergistic effect of morphology and molecules

Many authors (e.g. Wiens, 2004; Smith & Turner, 2005; Wahlberg *et al.*, 2005) have advocated the integration of morphological data with sequence data to yield a more robust phylogenetic hypothesis and recoup their value, e.g. for incorporating fossils in molecular phylogenies and in the search for more reliable morphological characters. Other authors (e.g.

Scotland *et al.*, 2003) see morphological data as inherently problematic and advocate simply mapping them on to molecular trees. In our case, adding 428 morphological characters for 139 exemplars among Afro-Malagasy tropical taxa of the '*Heteropsis* clade' did not greatly improve support for nodes of the '*Heteropsis* clade' based on ten genes (even reducing nodal support values for some nodes). It is otherwise apparent that the molecular sequence data tended to overwhelm the morphological data, containing relatively strong phylogenetic signal compared with the morphological data, at a wider range of levels (compare morphology-only phylogeny in File S4 with Figs 1–3). Although increasing the number of characters is generally considered advantageous for molecular data, this may not be the case for our morphological dataset. Taking into account the fact that homology assessment becomes increasingly difficult for large numbers of characters (Scotland *et al.*, 2003), we consider that the 428 morphological character matrix contains a mixture of homology and homoplasy, with the latter tending to overwhelm any phylogenetic signal, especially for the deeper relationships within the '*Heteropsis* clade' (File S4).

Conclusion

We have presented here a robust phylogenetic hypothesis for the relatively widespread palaeotropical '*Heteropsis* clade' which we confirm as monophyletic. We have defined this clade as comprising three well-defined, evolutionarily deep and strongly supported monophyletic lineages endemic to the regions of Madagascar, Africa, and Asia. We apply the generic names *Heteropsis*, *Brakefieldia* and *Telinga* to these groups, respectively. The proposed phylogenetic hypothesis confirms the closer affinity of *Brakefieldia* and *Telinga*, compared with *Heteropsis*. Based on the molecular evidence provided in this study, combined with morphological evidence, we recommend a number of new combinations and/or revised statuses within the '*Heteropsis* clade' for each genus (File S2).

The '*Heteropsis* clade' now presents an exciting opportunity and excellent model system for understanding the fundamental evolutionary mechanisms that operate on potentially similar temporal and spatial (archipelagic to continental scales). The relatively robust phylogenetic framework and potentially stable three-genus nomenclature for the group that we provide, along with recognition of the precise composition of the monophyletic species groups within each genus, will be indispensable for researchers working on the trait mapping of this group.

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/syen.12183

File S1. Table of vouchers and their accession numbers. In this table, morphospecies are given as numbers, which correspond to suffixes of the ms names in the trees Figures. 1–3, and letter codes correspond with the total-evidence-morphology tree in Lees (1997: 156). Species labelled 'ms' are names available in Lees, 2016. Exemplar taxa with names starting with 'xx_" and in black (e.g. xx_*Brakefieldia_elisi*) are taxa that were missed in the molecular phylogenies and here fit into the combined phylogeny using only morphological data, or combined with a very short fragment of some genes (*denotes taxa that were considered rogue in the molecular phylogenetic analyses).

File S2. Taxonomic details for the genera *Heteropsis*, *Brakefieldia* and *Telinga*.

File S3. Synonymic checklist for the '*Heteropsis* clade' encompassing all taxa in the genera *Heteropsis*, *Brakefieldia* and *Telinga*.

File S4. (MrBayes) Bayesian inference tree based on 428 morphological characters of the genera *Heteropsis*, *Brakefieldia* and *Telinga*. The colour-coded branches represent the three genera (blue, *Brakefieldia*; red, *Telinga*; green, *Heteropsis*) within the '*Heteropsis* clade'.

File S5. Details of ten primer-pairs used in the study and the thermal profile used of the polymerase chain reactions (PCRs).

Acknowledgements

We are grateful to Cletus Balangtaa, Bernard Turlin, António Bivar Sousa, Hitoshi Takano and Claire Kremen for providing us with valuable samples for the study. We thank Ghana Wildlife Division and Madagascar National Parks Association (PNM-ANGAP) for granting us permission to collect and export specimens in Ghana and Madagascar, respectively. We also thank the Kerala and Sikkim Forest Departments for collecting permits in India. A few photographs are copyright BMHN and taken by Harry Taylor; we thank the trustees of the Natural History Museum for permission to use these. Financial support for this study was provided by an ERC-EMARES (#250325) grant awarded to P.M. N.W. thanks the Academy of Finland for support. K.A.P. is currently supported by US NSF Grant (DEB-1541557) under the 'ButterflyNet' project.

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Accepted 29 February 2016
First published online 9 June 2016