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ORIGINAL ARTICLE

Recognition of a species-poor, geographically restricted but morphologically diverse Cape lineage of diving beetles (Coleoptera: Dytiscidae: Hyphydrini)

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ABSTRACT

Aim To establish the phylogeny and geographical origin of the genera of the diving beetle tribe Hyphydrini in order to investigate the origin of differences in geographical range size, intrageneric species-richness and morphological disparity. In particular, we tested the hypothesis that the geographically restricted, species-poor and morphologically deviating genera found in the Cape Region of South Africa are a paraphyletic pool of 'primitive' Hyphydrini, from which the morphologically more uniform, species-rich and geographically widespread genera have originated.

Location Worldwide, with special reference to the Cape Region of South Africa.

Methods We constructed a genus-level molecular phylogeny of 10 of the 14 known genera of Hyphydrini, including the five endemic to the Cape Region, using sequences from four gene fragments (two mitochondrial, rrnL and cox1; and two nuclear, 18S rRNA and histone 3, *c.* 2200 bp). Phylogenies were built with Bayesian methods, and linearized using penalized likelihood. Morphological disparity was characterized by correspondence analysis of a data matrix of 21 binary characters. We compare morphological disparity among groups using distance to the global and local centroids and the total range of morphospace occupied. Geographical range was estimated using the number of 6° longitude × 8° latitude Universal Transverse Mercator squares known to contain any species of each genus.

Results Hyphydrini is made up of four well supported clades of similar relative genetic divergence: (1) *Hyphydrus* (Old World plus Australasia, 133 species), (2) the five endemic genera of the Cape Region, sister to *Hovahydrus* (Madagascar) (10 species), (3) *Desmopachria* (America, 92 species), and (4) two Oriental genera (*Microdytes* and *Allopachria*, 68 species). The morphological disparity within the Cape Region lineage has apparently increased with time, with the two genera closest to the global centroid paraphyletic and basal with respect to the three more recent, morphologically deviating genera. Differences in the number of species between each of the four lineages were not significant. The correlation between the number of species in each lineage and geographical range extent was highly significant, with the low species number of the Cape Region (six) well within the 95% confidence interval of the regression.

Main conclusions Contrary to expectations, the species-poor, morphologically deviating endemic genera of the Cape Region are not a 'primitive' relictual pool from which the widespread, species-rich and morphologically uniform genera have originated. The morphological disparity within the Cape lineage has increased with time, and the apparent lack of species-level diversification disappears when species–area relationships are considered. A major unanswered

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question is why one of the four main lineages of Hyphydrini has remained restricted to a very reduced area (the Cape Region), but despite this evolved the highest degree of morphological diversity seen in the tribe.

Keywords

Adaptive radiation, Cape Region, Coleoptera, diversification, Dytiscidae, Hyphydrini, molecular phylogenies, morphological disparity, range size.

INTRODUCTION

The origin of the unequal distribution of species richness among lineages and geographical areas, and the potential links between these asymmetries and the origin of morphological and functional diversity, remains one of the major questions in evolutionary biology (Rosenzweig, 1995; Barton, 2001; Losos & Miles, 2002). Most efforts in this area have so far been devoted to the study of species-rich clades, either with high ecomorphological disparity ('evolutionary radiations', Schluter, 2000; Losos & Miles, 2002; Harmon *et al.*, 2003), or to non-adaptive radiations (lineages with a high phyletic diversity but relative ecomorphological uniformity, Gittenberger, 1991; Kozak *et al.*, 2006). In general, the study of radiations focuses on species-rich clades, considering species-poor lineages only by way of contrast. However, to understand the origin of differences in species number, morphological diversity or geographical range, it is necessary to study whole lineages with an unequal distribution of different combinations of these characteristics (Losos & Miles, 2002).

The diving beetles of the tribe Hyphydrini are a good example of such a group. As defined by Biström *et al.* (1997), Hyphydrini includes 14 genera with *c.* 300 species (Nilsson, 2001), although most of its species diversity is concentrated in a few morphologically very homogeneous genera with wide geographical distributions: *Desmopachria*, with *c.* 96 species in the Americas from Canada to central Chile; *Hyphydrus*, with *c.* 133 species and a cosmopolitan distribution with the exception of the Americas; and the *Microdytes–Allopachria* complex (see Results), with *c.* 68 species widely distributed in the Oriental and Eastern Palaearctic regions (Biström *et al.*, 1997; Nilsson, 2001; Table 1). In sharp contrast, there are five species-poor genera endemic to the Western Cape Region of South Africa: *Andex*, *Coelhydrus*, *Darwinhydrus*, *Primospes* and *Hydropeplus* (Biström *et al.*, 1997). The first four are monotypic, the last (*Hydropeplus*) has two species (Nilsson, 2001). Some of the endemic Cape species are relatively common, being known since the early 19th century (*Darwinhydrus*, for example, was described on the basis of specimens collected by Darwin in the Cape of Good Hope during the visit of HMS *Beagle* in 1836), but *Coelhydrus* and *Andex* are apparently rare and restricted in ecological range (Omer-Cooper, 1962; Toledo & Turner, 2004; Turner, 2004; Challet & Turner, 2006).

The endemic genera of the Cape Region are morphologically very distinct, and many of their unique characters have traditionally been interpreted as primitive: for example, body outline not fully streamlined, less adapted to swimming, slender legs, genitalia poorly differentiated (Guignot, 1961; Omer-Cooper, 1962). According to Sharp (1882: 928), the tribe Hyphydrini has its centre of origin in South Africa, with *Hyphydrus* (and other genera) being derived forms. The South African endemics have therefore usually been considered to be relicts of a very ancient Mesozoic sud-African fauna (Guignot, 1961: 928; Omer-Cooper, 1962), which could be equivalent to the traditionally recognized ancient, Gondwanan component of the Cape flora (Goldblatt, 1978; Linder, 2005). The clear-cut separation of Cape plant lineages into Cretaceous relicts of Gondwanan origin, elements of tropical African origin, and recent Palaearctic invasions has been challenged by several molecular phylogenies (for a review see Linder, 2005). These have revealed a more complex scenario, with incorporation of new lineages since the Cretaceous, but with multiple trans-continental colonizations after the breakdown of Gondwana and radiations of different ages, from late Oligocene to Plio-Pleistocene (Richardson *et al.*, 2001; Klak *et al.*, 2004; Linder & Hardy, 2004; Linder, 2005). Although most studies of the Cape flora have centred on species-rich clades (Richardson *et al.*, 2001; Linder, 2005), the region is certainly also home to some old, isolated lineages with few species, mostly associated with

Table 1 Genera of Hyphydrini, with number of species, main geographical distribution, estimated size of the combined geographical range (number of 6° × °8 UTM square grids; see Methods) and species included in the molecular phylogeny

Genus	No. of species	Distribution	UTM	Species studied
Andex	1	Cape Region	1	1
Coelhydrus	1	Cape Region	1	1
Darwinhydrus	1	Cape Region	1	1
Hydropeplus	2	Cape Region	1	1
Primospes	1	Cape Region	1	1
Hovahydrus	4	Madagascar	3	1
Hyphydrus	133	Oriental, Palaearctic, Ethiopian, Australian	120	7
Desmopachria	96	Nearctic, Neotropical	39	3
Agnoshydrus	5	Oriental	4	0
Allopachria	34	Oriental, E. Palaearctic	21	1
Anginopachria	3	Oriental	3	0
Dimitshydrus	1	Japan	1	0
Hyphovatus	3	Oriental	3	0
Microdytes	34	Oriental, E. Palaearctic	21	6

permanent streams and forests (Wishart & Day, 2002; Galley & Linder, 2006).

Here we use a comprehensive sample of Hyphydrini (including the five endemic Cape genera) to investigate the geographical origin of the tribe, and the origin of inequalities in geographical range size, species richness and morphological disparity. To do this we have constructed a robust genus-level molecular phylogeny of the tribe and compared the morphological disparity of the different lineages. In particular, we test the traditional hypothesis that considers the geographically restricted, species-poor and morphologically deviating genera of the Cape Region as a paraphyletic or polyphyletic pool of 'primitive' Hyphydrini, from within which the morphologically more specialized, species-rich and geographically widespread genera have originated.

This study of a Cape lineage of endemic aquatic beetles also contributes to our general understanding of the evolution of the Cape biota by introducing a novel taxonomic group with deviating characteristics (low species richness), examined in the context of a well sampled, well defined extra-Cape lineage. As noted by Barraclough (2006), to explain the high diversity of the Cape flora we need to compare the rates and mode of evolutionary processes in the region with those observed elsewhere.

Background on the phylogeny of Hyphidrini

The phylogenetic position of Hyphydrini within the dytiscid subfamily Hydroporinae is not well established. Biström *et al.* (1997) hypothesize that it is sister to Pachydrini, traditionally included within Hyphydrini. Miller (2001a) (who did not include any of the endemic Cape genera in his analyses) merged the two tribes again (followed by Nilsson, 2001) – although, based on molecular data (Ribera *et al.*, 2002; unpublished data; this study), the two groups are not closely related. Alarie *et al.* (1997) included the known Hyphydrini larvae in a phylogenetic analysis, in which they were found to be related to Hydroporini (including Hygrotini) and Bidessini. According to Alarie *et al.* (1997) and Alarie & Challet (2006a,b), the monophyly of Hyphydrini plus *Pachydrus* is strongly supported, with *Pachydrus* considered to be an 'old' lineage with many plesiomorphic characters. Michat & Torres (2005) found Hyphydrini to be related to Vatellini and Bidessini, based on larval characters.

In the only comprehensive formal phylogeny of the tribe, Biström *et al.* (1997) obtained an almost fully unresolved tree, with a monophyletic Hyphydrini, but with only two monophyletic genera, *Allopachria* and *Desmopachria*. They could not find evidence for the monophyly of *Hyphydrus* and *Microdytes*, which were divided in several independent lineages. Although no synapomorphy could be found, the genera *Microdytes* and *Allopachria* were hypothesized to be related, with only one important structural difference (prosternal process dentate in *Microdytes*, not dentate in *Allopachria*). Alarie & Challet (2006a,b) described the larvae of *Primospes* and *Andex*, respectively, with a phylogenetic analysis of the known larvae of Hyphydrini. *Pachydrus* was found to be the sister of Hyphydrini, and the two Cape genera were paraphyletic with respect to *Desmopachria* + *Hyphydrus*, in agreement with the traditional view of the paraphyly of the Cape endemic genera.

METHODS

Sampling

For the molecular phylogeny, we included representatives of all known Hyphydrini genera except four (Table 1; Appendix S1 in Supplementary Material): (1) *Anginopachria*, a recently described genus with three species from Southeast Asia, originally described within *Allopachria*, (2) *Agnoshydrus*, with five Oriental species, related to *Allopachria* and *Microdytes* (the type species of *Agnoshydrus* has been successively included in both *Allopachria* and *Microdytes*), (3) *Hyphovatus*, with three Oriental species superficially close to *Hyphydrus* and *Hovahydrus*, different from *Allopachria* and *Microdytes* only in the shape of the parameres (Biström *et al.*, 1997), and (4) *Dimitshydrus*, with a single stygobiontic species from Japan, close to *Microdytes* and *Allopachria*, although apparently linked to *Hyphydrus* on some structural characters (Uéno, 1996).

In order to discount the existence of cryptic species within the species-poor Cape Region genera, efforts were made to include more than one specimen, despite the general rarity of some of the species (Turner, 2004; Challet & Turner, 2006). Two specimens of *Andex insignis* Sharp were included in the analyses, and a partial sequence of an additional specimen of *Hydropeplus trimaculatus* (Laporte) was obtained (not included in the final analyses) (Appendix S1).

For measurement of morphological disparity, the genera *Agnoshydrus*, *Hyphovatus* and *Anginopachria* were also included, the first two as originally studied by Biström *et al.* (1997), the latter newly coded for the same characters (see below).

We used a wide representation of the main tribes of Hydroporini as outgroups, including Pachydrini, Hygrotini, Hydroporini, Laccornini, Hydrovatini, Bidessini and Vatellini (Nilsson, 2001; Ribera *et al.*, 2002) (Appendix S1). The tree was rooted in Laccophilinae, assumed to be closely related to Hydroporinae (Miller, 2001a; unpublished results).

DNA extraction and sequencing

Genomic DNA was obtained from ethanol-preserved specimens through a standard non-destructive phenol–chloroform extraction or with extraction columns (see Appendix S1 for specimen details and GenBank accession numbers). Four gene fragments were sequenced, two mitochondrial (3′ end of the rrnL; 3′ end of cox1) and two nuclear (5′ end of 18S rRNA, a fragment of histone 3). The same markers have been used previously in diving beetles, and provided good phylogenetic resolution (Ribera *et al.*, 2002; Ribera & Vogler, 2004; Balke *et al.*, 2005). Both forward and reverse sequences were obtained for each fragment. Primers used and sequencing

conditions were the same as in previous studies of the same family (Ribera *et al.*, 2002; Balke *et al.*, 2005). New sequences were submitted to GenBank with accession numbers EF056547–EF056700 and EF059805.

Phylogenetic analyses

We followed a two-step procedure for the alignment of length-variable regions, with an initial hypothesis of character homology (an alignment) and a subsequent, independent step of tree search (Phillips *et al.*, 2000; Simmons, 2004).

The optimal model of nucleotide substitution was determined with MODELTEST 3.6 (Posada & Crandall, 1998), for all genes separately and for the combined data set. We analysed the combined data set using Bayesian probabilities (Rannala & Yang, 1996) as implemented in the computer program MRBAYES 3.0b4 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003), as it allows the independent estimation of different parameters of the evolutionary model for the user-defined data partitions. Searches were conducted using the default parameters, starting with random trees, with three heated and one cold Markov chains for 1,000,000 generations, sampled at intervals of 100 generations. To determine the point at which the Markov chains reached stationarity, the log-likelihood scores were plotted against generation time, and determined visually when the log-likelihood values reached a stable equilibrium. The parameter estimations (including tree topologies) obtained before reaching stationarity were discarded as a 'burn-in', and only the trees sampled after that point were considered (Huelsenbeck & Ronquist, 2001). Two independent runs were conducted to check for convergence and to avoid being trapped in local optima (Huelsenbeck & Ronquist, 2001).

Posterior probabilities were used to assess node stability. Although generally higher than bootstrap support values, posterior probabilities above the standard 95% threshold can be taken as indicative of strong node stability (Suzuki *et al.*, 2002; Alfaro *et al.*, 2003; Douady *et al.*, 2003; Simmons *et al.*, 2004). Posterior probabilities can be taken as the probability that a node is true, provided that the data evolve according to the model used (Huelsenbeck & Rannala, 2004).

For comparative purposes, additional maximum likelihood searches were conducted with the program PHYML (Guindon & Gascuel, 2003), implementing the optimal combined model and starting with a neighbour-joining tree. Node support was measured with 100 bootstrap replicates. Parsimony analyses were conducted under equal weights in PAUP* ver. 4.0b10 (Swofford, 2002), using tree bisection–reconnection (TBR) heuristic searches with 1000 random addition sequences and without saving multiple trees. A heuristic search was subsequently conducted on the most parsimonious trees found, with the option 'save multiple trees' activated, until the end of the search, or until more than 5000 trees were found. In all searches, gaps were coded as missing data. Node support was measured with non-parametric bootstrapping with 1000 pseudoreplicates of 50 random additions each (Felsenstein, 1985).

To try and establish the phylogenetic position of the genera for which no molecular data were available, we used the morphological matrix of Biström *et al.* (1997) (with the addition of *Anginopachria*) in combination with the molecular data. When the morphological data were analysed separately, the parsimony search resulted in an almost fully unresolved tree (not shown; see also Biström *et al.*, 1997). We thus used the preferred molecular tree (see Results) as a backbone constraint, and performed heuristic parsimony searches with the morphological matrix.

Estimation of rates of variation

To estimate the relative time of divergence, we used the topology and branch lengths of the tree obtained using the combined data in MRBAYES (see Results), with the exclusion of the long branch leading to the New Guinean *Hyphydrus*. The possibility of directly enforcing a molecular clock to the data was tested using likelihood ratios in PAUP: the likelihood of the tree under the optimal model and using the parameters estimated in MRBAYES was computed, and compared with the likelihood when enforcing a molecular clock. As clock-like behaviour of the data was significantly rejected (see Results), we used the penalized likelihood (PL) method of Sanderson (2002) to obtain an ultrametric tree, as implemented in the software program r8s (http://ginger.ucdavis.edu/r8s). The smoothing parameter was determined by a process of cross-validation in a preliminary analysis using the truncated Newton (TN) method, and a second analysis was run with the smoothing parameter set to the optimal value obtained previously (Sanderson, 2002). It must be noted that the whole molecular-clock approach is subject to a large number of uncertainties (for a review see Bromham & Penny, 2003), but our purpose was only to check the relative age of the four main nodes.

To compare the rate of diversification between sister lineages, we used the test of Slowinski & Guyer (1993). This is a very conservative test (Kirkpatrick & Slatkin, 1993), with a high type 2 error, but other, more powerful measures of tree inequality (e.g. Kirkpatrick & Slatkin, 1993) could not be applied due to the incomplete sampling within the more diverse genera (*Hyphydrus*, *Desmopachria*, *Microdytes* and *Allopachria*).

Morphological disparity

To estimate morphological disparity, we used the character matrix provided by Biström *et al.* (1997) with the addition of *Anginopachria* (described by Wewalka *et al.*, 2001), coded for the same characters (Appendices S2 & S3). We could not obtain specimens of *Dimitshydrus* for study.

The characters coded in the matrix were originally intended to be used in a phylogenetic context, and thus reflect structural variation rather than quantitative differences in body shape or

size. The authors explicitly address the monophyly of the genera, including intra-generic variation that could be of less relevance for the deep phylogeny of the tribe, but that could reveal the non-monophyly of some of the taxa. The whole range of variation within the genera was thus studied by Biström *et al.* (1997), and some divided into several terminal taxa when they were found to not be homogeneous for all characters (Biström *et al.*, 1997; Appendices S2 & S3). Characters 13, 14, 15, 18 and 24 of the original matrix were not variable within the ingroup (Hyphydrini), and were therefore deleted from the matrix. The final data set included 21 binary characters for 19 terminal taxa (Appendices S2 & S3). Characters 20 and 23 of Biström *et al.* (1997) could not be examined for *Anginopachria*, and were assigned the same character state as the related genera *Microdytes* and *Allopachria* (see Results).

The morphospace defined by the species of Hyphydrini was characterized through correspondence analyses, a multivariate method well suited to summarize a qualitative binary matrix (Legendre & Legendre, 1998). To quantify the morphological disparity, we computed the centroid (average) and range of the scores for each axis, for each of the groups to be compared (Foote, 1992). Following Foote (1992), we used the sum of ranges as opposed to the hypervolume (multiplied ranges), because it allows each axis to contribute to the total range in proportion to the amount of variance it explains (Wills, 2001). The morphospace volume, taken as range, is known to be strongly influenced by the number of species in the sample and the presence of outliers (Foote, 1992), so comparisons were made using both measures (distance to centroid and total range). In any case, it must be stressed that the sampling of the morphological disparity of the group is exhaustive: all known genera, subgenera and species groups were studied and their morphological characters summarized in the matrix presented by Biström *et al.* (1997).

In the character matrix of Biström *et al.* (1997), the terminal taxa represent different numbers of species. We repeated the analyses using all species of Hyphydrini (as in Table 1), using duplicated entries for species with the same distribution of character states. Results (not shown) were essentially the same, increasing the range of scores in a non-linear way but keeping the relative position of the taxa.

Relationship between size of geographical range and number of species

To estimate the size of the geographical range of the main clades, the Earth's surface was divided into Universal Transverse Mercator (UTM) grid squares of 6° longitude and 8° latitude. The number of squares occupied by any of the species of each genus was recorded (discarding grid squares lying mostly in the sea). We computed the linear regression through the origin between the logarithm of the number of species vs. the logarithm of the number of UTM grid squares occupied for each of the target clades.

RESULTS

Phylogenetic analyses

The final data matrix had 2206 characters, of which 635 were informative. Protein-coding genes (*cox1* and *histone3*) were not length-variable, and the number of indels among the ingroup was minimal and easily alignable: for 18S rRNA a two-base insertion in *Desmopachria* sp1, some insertions (1,1,4,2,2) in *Hovahydrus*, and one base insertion in *Hyphydrus ovatus* (L.). For the rrnL, maximum length differences were of five nucleotides, distributed in different indels. The two specimens of *A. insignis* had identical ribosomal sequences, and differed in <1.0 and <1.5% in *H3* and *cox1*, respectively. The partial *cox1* sequence of the second specimen of *H. trimaculatus* was of poor quality (and not included in the analyses), but had <2% unambiguous changes.

The optimal evolutionary model, as estimated by MODELTEST, included rate heterogeneity (G) and a proportion of invariant sites (I) for all genes (Yang, 1993). The number of parameters of the transition matrix were different for the four genes, and the more complex model (generalized time-reversible or GTR; Tavaré, 1986) was implemented. The two runs resulted in identical topologies (Fig. 1). The monophyly of Hyphydrini was strongly supported, and the tribe found to be sister to Hygrotini (although with low support). The genus *Pachydrus*, traditionally assumed to be related to Hyphydrini (or included among them; Miller, 2001a) was found to be sister to Bidessini, with strong support (Fig. 1).

All the genera endemic to the Cape Region were grouped in a monophyletic lineage, sister to the Malgasian *Hovahydrus*. These two lineages were, in turn, sister to *Hyphydrus*. The genus *Desmopachria* (America; Table 1) was sister to an Oriental clade (*Allopachria* plus *Microdytes*), and both were sister to *Hyphydrus* plus the African genera. The studied species of *Allopachria* was placed within *Microdytes*, suggesting the possibility that the two genera should be considered synonyms.

The use of the same matrix for maximum likelihood (ML) searches in PHYML, implementing the same evolutionary model (GTR + I + G) for the whole sequence, and the heuristic parsimony searches resulted in very similar topologies. Only the node defining the relationship of *Andex* + *Hydropeplus* with *Darwinhydrus* and *Primospes* had a different topology in the ML tree, although with low support (*Darwinhydrus* sister to *Primospes*, bootstrap 68%) (Fig. 1).

Rate variation

The clock-like variation of the sequences was significantly rejected (even after the exclusion of the long branch leading to the New Guinean *Hyphydrus*; Fig. 1), as measured with likelihood ratios using the topology and branch length obtained in MRBAYES, and the evolutionary model GTR + I + G ($P < 0.001$). In the ultrametric tree obtained implementing the PL method of Sanderson (2002) (with an

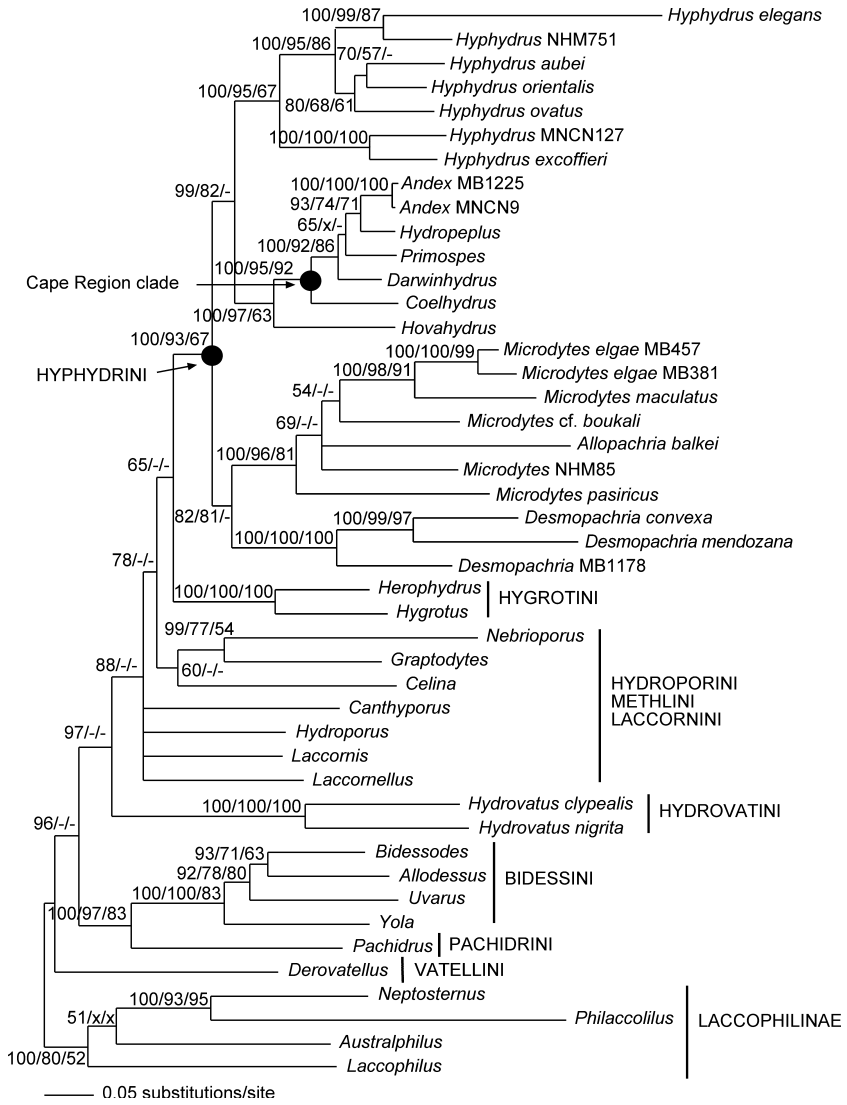

Figure 1 Phylogeny of Hyphydrini as estimated with Bayesian methods. Numbers in nodes, Bayesian posterior probabilities (×100)/bootstrap maximum likelihood (ML) values (as computed in PHYML)/bootstrap parsimony values (see Methods for details). Only values above 50% are included. When a value is not included, the corresponding node was either present with lower support or unresolved. Contradictory nodes are marked 'x' (for the ingroup, only the position of *Hydropeplus* in the ML tree, see Results).

estimated optimal smoothing parameter of 39.81), the four main clades appeared remarkably symmetrical, with a similar estimated divergence for the basal nodes (Fig. 2). Thus the divergence among the genera of the Cape Region clade appears to be within the range of that seen in the large hyphydrine genera (*Desmopachria*, *Hyphydrus*, or *Microdytes* + *Allopachria*).

Phylogenetic placement of the missing genera

When the morphological matrix of Biström *et al.* (1997) (plus *Anginopachria*) was analysed using parsimony, enforcing the Bayesian molecular topology as a backbone constraint, the three genera with missing molecular data (*Agnoshydrus*, *Hyphovatus* and *Anginopachria*) were included in the *Desmopachria* plus *Allopachria* + *Microdytes* clade (with *Anginopachria* sister to *Agnoshydrus*), to the exclusion of the clade formed by the Cape Region genera, *Hovahydrus* and *Hyphydrus*.

Morphological disparity

The first six axes of the correspondence analysis on the morphological matrix had an accumulated variance of 82% (Table 2), and were considered further for the comparisons of morphological disparity. Additional axes each included <5% of total variance, and were mostly correlated with single variables.

The five endemic genera of the Cape lineage occupied extreme positions in the morphospace defined by the Euclidean distance to the global centroid (Fig. 3). The two older genera (*Darwinhydrus* and *Coelhydrus*) had the shortest distances (were almost perfect 'average' Hyphydrini), while the three monophyletic, most recently derived genera (*Andex*, *Primospes* and *Hydropeplus*) had the longest distances (the most deviating morphologies).

In accordance with the phylogenetic results (see above), the Cape Region clade plus *Hovahydrus* (six terminal taxa in the morphological matrix) was compared with its sister, *Hyphydrus* (three terminal taxa). Three additional comparisons were made

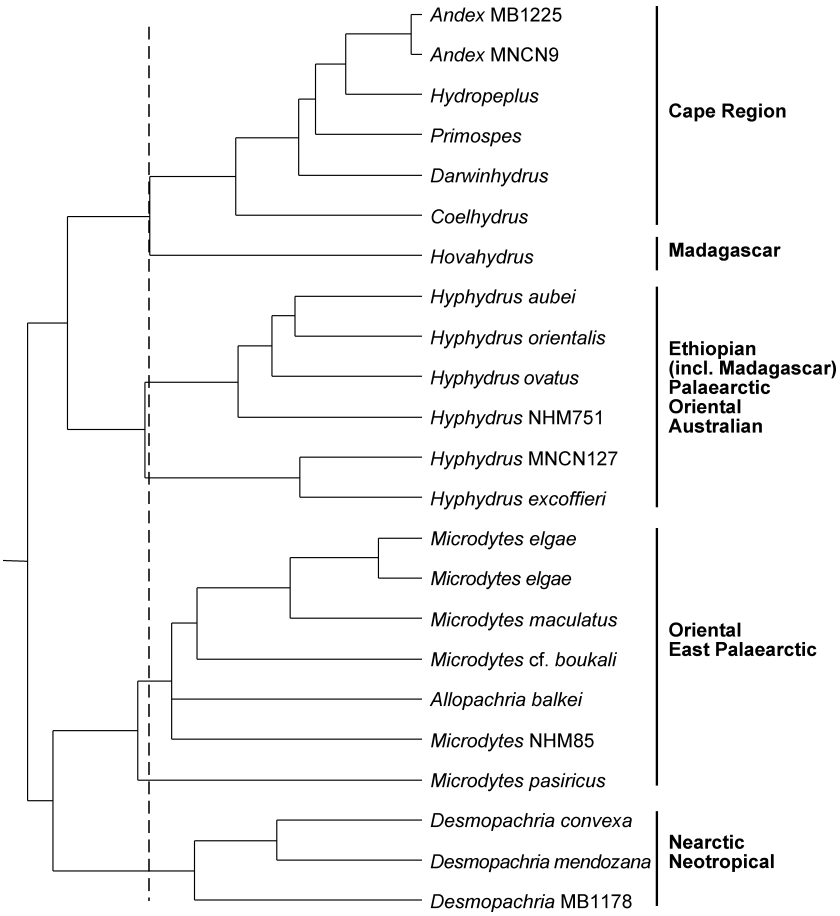

Figure 2 Ultrametric tree of Hyphydrini estimated through penalized likelihood (Sanderson, 2002) using the topology and branch lengths of the Bayesian probability tree in Fig. 1 (see Methods). Note the similar estimated relative coalescent age for the four main clades (dashed line).

in an attempt to localize more precisely the differences in morphological disparity between the groups. These comparisons were not between sister taxa, as they include a monophyletic clade vs. a paraphyletic clade including its sister, but in all cases the potential bias runs against the hypothesis of higher morphological disparity among the Cape Region clade: (2) Cape clade vs. (*Hovahydrus* + *Hyphydrus*), (3) Cape clade excluding *Coelhydrus* vs. (*Coelhydrus* + *Hovahydrus* + *Hyphydrus*), (4) (*Andex* + *Hydropeplus* + *Primospes*) vs. (*Darwinhydrus* + *Coelhydrus* + *Hovahydrus* + *Hyphydrus*) (Table 3). In all cases, the group including the most recent Cape Region endemics had a larger total morphological disparity (as measured with the sum of the ranges of the six axes) and a longer average Euclidean distance to the local centroid (Table 3).

Geographical range vs. number of species

The diversification rate of the two main sister lineages within Hyphydrini, and of the two sister clades in each of the main lineages, could not be considered significantly different according to the test of Slowinski & Guyer (1993) ($P >> 0.1$).

The regression through the origin of the logarithm of the number of species vs. the logarithm of the area occupied for the three main Hyphydrini clades plus *Hovahydrus* and the Cape clade was highly significant ($r^2 = 0.90$, $P < 0.005$, $n = 5$) (Fig. 4). The residual of the Cape clade was the highest (+0.78), although within the 95% confidence interval of the regression (±1.18) (Fig. 4).

DISCUSSION

Phylogeny of Hyphydrini

Contrary to expectations (Sharp, 1882; Guignot, 1961; Alarie & Challet, 2006b), the endemic genera of Hyphydrini from the Cape Region form a monophyletic clade, sister to the endemic genus from Madagascar (*Hovahydrus*) and both sister to a widespread and species-rich genus of probable African origin (*Hyphydrus*, Biström, 1982a). The expected pattern of 'progress' within the tribe from a paraphyletic pool of less adapted, less diversified Cape Region genera to a reduced number of highly adapted and widespread species-rich genera was thus reversed, resulting in a species-poor, geographically restricted lineage that shows high morphological diversity.

The sister relationship between the Cape lineage and *Hovahydrus*, endemic to Madagascar, parallels the frequent relationship of African and Malagasian insects (Goodman & Benstead, 2003; Monaghan *et al.*, 2005), suggesting dispersal from mainland Africa as a possible origin for *Hovahydrus* (as opposed to a vicariant origin, which would require a sister relationship of *Hovahydrus* with the Oriental clade; Sanmartín

Table 2 Scores for the first six axes of the correspondence analysis of the morphological matrix (see Supplementary Material)

Parameter/ species	Axis 1	2	3	4	5	6
Eigenvalue	0.71	0.60	0.44	0.31	0.25	0.16
Inertia (%)	24	20	15	10	8	5
Cumulative (%)	24	43	58	68	77	82
Andex	0.05	2.60	−0.33	0.61	0.15	0.31
Coelhydrus	−0.61	0.17	−0.01	0.07	0.14	0.00
Darwinhydrus	0.05	0.22	0.67	−0.21	−0.57	−0.13
Hydropeplus	−0.11	2.53	0.17	1.84	−0.26	−0.96
Primospes	0.10	1.76	−0.41	−1.06	0.33	1.46
Microdytes	0.28	−0.32	0.82	−0.15	−0.85	−0.34
Allopachria 1	1.24	−0.36	−0.36	−0.30	−0.56	−0.86
Allopachria 2	1.67	−0.63	−0.46	0.59	−0.25	0.30
Allopachria 3	1.64	−0.61	−0.08	0.60	0.36	0.37
Agnoshydrus	0.57	0.04	0.18	−0.28	−0.59	−0.54
Hyphovatus	0.46	−0.01	1.67	−0.19	1.35	−0.13
Hovahydrus	−0.17	0.07	0.73	−0.25	−0.74	0.07
Hyphydrus 1	−0.33	−0.04	0.65	−0.22	−1.08	0.65
Hyphydrus 2	−0.06	0.06	1.43	−0.44	0.23	−0.19
Hyphydrus 3	−0.17	0.10	0.73	−0.25	−0.74	0.07
Desmopachria 1	−0.77	−0.38	−0.32	0.13	0.19	−0.10
Desmopachria 2	−0.80	−0.40	−0.35	0.16	0.22	−0.08
Desmopachria 3	−0.80	−0.37	−0.29	0.14	0.03	0.13
Anginopachria	0.66	0.58	−1.03	−1.28	0.24	−0.37

& Ronquist, 2004). This is also contrary to the reconstructed origin of most of the Cape clades of plants, which tend to have a trans-Indian Ocean origin (although some are also sister to clades of African origin; Galley & Linder, 2006).

Our phylogenetic results may call into question the taxonomic treatment of the Cape Region clade as five distinct genera instead of a single one. We prefer to maintain the current treatment, however, to favour stability and to recognize their morphological distinctiveness.

Geographical range size, number of species and morphological disparity

Despite the apparent differences in species numbers between the four main lineages of Hyphydrini (Fig. 5), none of the nodes in the reconstructed phylogeny shows any significant difference in the number of species, as measured using the test proposed by Slowinski & Guyer (1993). This test is admittedly conservative (Kirkpatrick & Slatkin, 1993; Slowinski & Guyer, 1993), but the possibility certainly exists that random differences in early stages of the lineages lead to dramatic differences in the final number of species. It could be argued that there is likely to be a strong bias in the number of undescribed species: the Cape Region is a relatively well known area (but cf. Ribera *et al.*, 2002), and five of the six currently recognized species of the Cape clade were known by the late

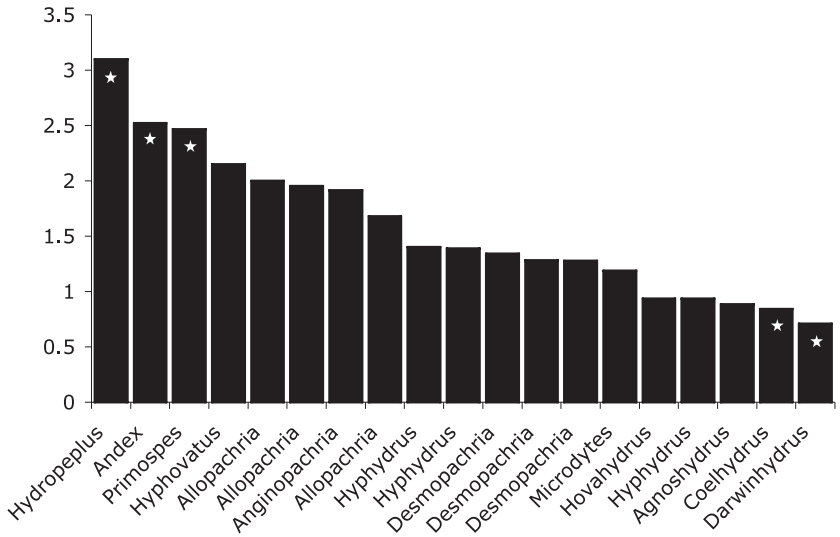

Figure 3 Euclidean distance to the global centroid of the genera of Hyphydrini in the first six axes of the correspondence analysis of the morphological matrix, sorted by decreasing magnitude. White stars, genera endemic to the Cape Region.

Table 3 Comparison of range of morphospace and distance to respective centroids for different combinations of taxa within the Cape Region endemic genera plus *Hovahydrus* + *Hyphydrus* (see Fig. 1)

A	No. *a*	ran	cen	*b*	No. *b*	ran	cen
AN + HD + PR + DA + CO + HO	6	1.35	1.67	HP	3	0.37	0.71
AN + HD + PR + DA + CO	5	1.30	1.70	HP + HO	4	0.37	0.57
AN + HD + PR + DA	4	1.17	1.72	HP + HO + CO	5	0.59	0.72
AN + HD + PR	3	0.76	1.45	HP + HO + CO + DA	6	0.63	0.66

ran, Sum of ranges; cen, centroid. AN, *Andex*; HD, *Hydropeplus*; PR, *Primospes*; DA, *Darwinhydrus*; CO, *Coelhydrus*; HO, *Hovahydrus*; HP, *Hyphydrus*.

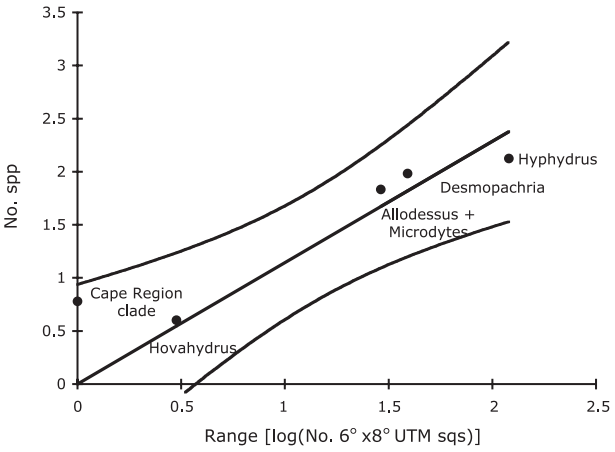

Figure 4 Regression through the origin between the logarithm of the number of species and the logarithm of the combined geographical range of the main clades of Hyphydrini, with 95% confidence interval for the regression values (measured as number of occupied UTM squares of 6° × °8; see Methods) ($n = 5$, $r^2 = 0.90$, $P < 0.005$).

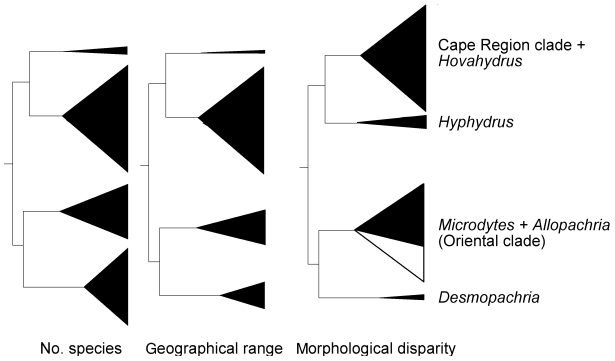

Figure 5 Comparison between number of species, geographical range and morphological disparity of the four main clades of Hyphydrini. The vertical dimension of the clade is proportional to the respective magnitudes (the size of the largest is the same for the tree measures, to facilitate comparison); the horizontal extension is proportional to the genetic divergence (as estimated from the linearized tree of Fig. 2, see Results). Morphological disparity is measured as the range of the first six axes of the correspondence analysis. The white portion of the morphological disparity corresponds to the inclusion of *Anginopachria*, *Agnoshydrus* and *Hyphovatus* (for which there are no genetic data) in the Oriental clade.

19th century (Sharp, 1882) (the last was described by Omer-Cooper, 1965). Based on the scarce genetic information available, the existence of a large number of unrecognized cryptic taxa among the Cape species does not seem likely. In contrast to the endemic Cape groups, only 25 species of *Hyphydrus* and 13 of *Desmopachria* were known to Sharp (1882). In the four most speciose genera, more than 75 species have been described in the past decade, and certainly many more await description. Despite the large bias in our knowledge of the diversity of the different genera of Hyphydrini, the difference in the number of species between two sister lineages needed to make the test of Slowinski & Guyer (1993) significant (40-fold) is likely to be too large to meet.

The difference in species numbers between lineages of Hyphydrini takes on a different light when viewed in the context of the size of their geographical distributions. The highly significant correlation between the number of species in each monophyletic lineage and the combined area of their geographical ranges (only slightly smaller when the relative depth of the nodes is taken into account; data not shown) suggests a non-random pattern, with no decrease in the number of species/area in the Cape Region – or even an increase. When the Cape clade was excluded from the regression ($r^2 = 0.94$, $P < 0.005$, $n = 4$), the 95% confidence interval for the extrapolation to the origin was ±0.60, corresponding to ±4 species, and lower than the actual richness of the Cape clade (0.78, or six species). The standard approach to the species–area relationship is to count the number of unrelated (non-monophyletic) taxa in well defined, non-overlapping geographical units (Rosenzweig, 1995; Lomolino, 2000; but cf. Losos & Schluter, 2000). Here we use the number of species in non-overlapping, well defined monophyletic clades vs. their cumulative geographical range, no matter whether discontinuous or overlapping among lineages. It will be interesting to see if the high correlation found among the lineages of Hyphydrini is a common pattern found in other organisms and taxonomic levels. The causal relationships, if any, could be in both directions: the area could restrict the diversification rate, in that a lineage confined to a certain area (defined through ecological, topographical or other parameters) may have its diversification limited; or a lineage with a given diversification rate may have a limited capability to expand its global geographic range. However, considering constraints in the potential size of the geographical range of a clade as a limiting factor in its diversification rate would run against what seems to be the common pattern in the Cape flora (or other Mediterranean areas), where a high number of closely related species are packed into a small area (Cowling *et al.*, 1996; Linder, 2005).

The morphological data used to estimate disparity in Hyphydrini lineages were compiled specifically to reflect structural differences with phylogenetic value (Biström *et al.*, 1997). They were also based on the examination of all genera and species groups known at the time, and thus can be considered a good representation of the actual morphological disparity of the tribe. Although the more speciose genera were found to be heterogeneous with respect to some of the characters studied, the morphospace they define is still substantially less diverse than that formed by the species-poor Cape clade (Fig. 5). The high morphological homogeneity of the two more speciose, widely distributed genera is reflected in their taxonomic treatment: despite various attempts to establish subgenera to partition their large diversity (e.g. Young, 1980 for *Desmopachria*; Guignot, 1959 for *Hyphydrus*), they are currently considered to be only conventional groupings

without any phylogenetic value (Biström, 1982a; Miller, 2001b, respectively). The species of all four most speciose genera are very similar in their external morphology, in both adults and larvae, and are often distinguishable only by the examination of internal genital characters (Biström, 1982a; Wewalka, 1997, 2000; Miller, 2001b). The Oriental clade seems to be morphologically more diverse than *Hyphydrus* or *Desmopachria* (Fig. 5), mostly due to the inclusion of the genera *Anginopachria* and *Allopachria* (Fig. 3). The uncertainties in the phylogenetic placement of the latter genera within the Oriental clade did not allow a more detailed analysis of these differences.

Our phylogenetic results show four main lineages within Hyphydrini, all having species with the derived morphology considered to be typical of the group (Biström *et al.*, 1997): a very convex, almost spherical body shape, long and slender legs, and a continuous outline, considered to be an adaptation to high manoeuvrability (Ribera & Nilsson, 1995; Ribera *et al.*, 1997). This 'ground plan' can also be recognized in the species of *Hovahydrus* from Madagascar, with four recognized species originally described within *Hyphydrus*. The species of *Hovahydrus* are morphologically similar to *Hyphydrus*, to which they were formerly considered closely related, but are smaller in size (Biström, 1982b).

Our results show that morphological disparity increases in the more derived species of the Cape clade, *Andex*, *Hydropeplus* and *Primospes*. When the Euclidean distances to the global centroid (the centroid of the morphospace defined by the whole Hyphydrini) were computed, the three largest were those of these three genera, while the distances of *Coelhydrus* and *Darwinhydrus* were the smallest (Fig. 3). The trend of increasing morphological disparity in the Cape Region clade is contrary to what seems to be the general pattern of adaptive radiations: an early origin of ecomorphological diversity plus long-term persistence of among-lineage differences (Foote, 1997; Schluter, 2000; Harmon *et al.*, 2003; Kozak *et al.*, 2005; Vitt & Pianka, 2005), although there are also some known cases of late diversification (e.g. Foote, 1997). The standard interpretation of the increase of ecomorphological disparity is to link it with the build-up and maintenance of species richness (Kozak *et al.*, 2005). Ecomorphological divergence is assumed to play a key role in the diversification, long-term persistence and co-existence of lineages by reducing the strength of ecological interactions among species (Simpson, 1953; Schluter, 2000; Kozak *et al.*, 2005). As noted above, although in absolute terms the Cape lineage of Hyphydrini is species-poor in comparison with its sister (but not significantly poorer), when considering the size of the geographical range the number of species per area is equivalent to, or higher than, that of the most species-rich genera. To test the possibility that the increase in morphological disparity observed in the most recent lineages of the Cape clade could be due to the same mechanism operating in species-rich radiations (favouring co-existence by reducing interactions) would require more data on the ecology and natural history of these rare species.

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SUPPLEMENTARY MATERIAL

The following supplementary material is available for this article:

Appendix S1 Studied material, with GenBank accession numbers of the sequenced genes.

Appendix S2 Data matrix for the correspondence analysis (from Biström *et al.*, 1997 with the addition of *Anginopachria*).
Appendix S3 List of characters used in the correspondence analysis. Numeration follows Biström *et al.* (1997).

This material is available as part of the online article from: http://www.blackwell-synergy.com/doi/abs/10.1111/j.1365-2699.2007.01694.x

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BIOSKETCHES

Ignacio Ribera investigates speciation and diversification processes in Coleoptera, with special emphasis on the systematics and biogeography of aquatic and subterraneous beetles.

Michael Balke is interested in the origin and evolution of the Southeast Asian and Australasian fauna of water beetles, and in the systematics of the Dytiscidae.

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Supplement 1
Studied material, with Genbank accession numbers of the sequenced genes. Nomenclature follows Nilsson (2001) except for the consideration of Pachidrini as a separate tribe. Voucher depositories: NHM, Natural History Museum (London); MNCN, Museo Nacional de Ciencias Naturales (Madrid); MB, M. Balke coll. (Zoologische Staatssammlung, München) (*, voucher deposited in the collection of G. Challet, California).

No.	Voucher ref.	Species	Tribe	rrnL	cox1	SSU	H3	Country	Locality	Collector
1	NHM-IR746	*Allopachria balkei*	Hyphydrini	EF056663	EF056592	EF056631	EF056548	Malaysia	Sabah	F. Ciampor
2	MB1225*	*Andex insignis*	Hyphydrini	EF056664	EF056593	EF056632	EF056549	South Africa	North Cape, E Garies	G. Challet
3	MNCN-AI9	*Andex insignis*	Hyphydrini	EF056665	EF059805	EF056633	EF056550	South Africa	North Cape, Studer Pass	G. Challet
4	MB1224*	*Coelhydrus brevicollis*	Hyphydrini	EF056670	EF056598	EF056635	EF056555	South Africa	West Cape, Lamberts Bay	G. Challet
5	MB354	*Darwinhydrus solidus*	Hyphydrini	EF056671	EF056599	EF056636	EF056556	South Africa	Mitchells Pass, Ceres	D.T. Bilton
6	NHM-IR573	*Desmopachria convexa*	Hyphydrini	EF056673	EF056601	EF056638	EF056558	US	Vermont, Bennington	C. Hernando
7	NHM-IR118	*Desmopachria mendozana*	Hyphydrini	EF056674	EF056602	EF056639	EF056559	Chile	IX Reg., Gorbea	I. Ribera
8	MB1178	*Desmopachria* sp	Hyphydrini	EF056675	EF056603	EF056640	EF056560	Venezuela	Amazonas, Puerto Ayacucho	M. Balke
9	MNCN-AI126	*Hovahydrus* sp	Hyphydrini	EF056677	EF056606	EF056642	EF056563	Madagascar	Andasibe, Forestry Station	M. Balke
10	MB355	*Hydropeplus trimaculatus*	Hyphydrini	EF056678	EF056608	EF056643	EF056565	South Africa	Mitchells Pass, Ceres	D.T. Bilton
11	MNCN-AI6	*Hydropeplus trimaculatus*	Hyphydrini	-	EF056607	-	-	South Africa	W Cape, Table Mountain	C. R. Turner
12	NHM-IR70	*Hyphydrus aubei*	Hyphydrini	EF056687	EF056617	EF056650	EF056575	Spain	Albacete	I. Ribera
13	MB388	*Hyphydrus elegans*	Hyphydrini	EF056684	EF056614	EF056647	EF056572	Papua New Guinea	EHL, Aiyura	K. Sagata
14	MNCN-AI93	*Hyphydrus excoffieri*	Hyphydrini	EF056682	EF056612	EF056645	EF056570	China	Yunnan, Shizong	J. Bergsten
15	MB1236	*Hyphydrus orientalis*	Hyphydrini	EF056683	EF056613	EF056646	EF056571	China	Yunnan, Shizong	J. Bergsten
16	NHM-IR15	*Hyphydrus ovatus*	Hyphydrini	EF056688	EF056618	EF056651	EF056576	UK	Sommerset Levels	I. Ribera
17	NHM-IR751	*Hyphydrus* sp1	Hyphydrini	EF056685	EF056615	EF056648	EF056573	Malaysia	Sabah	F. Ciampor
18	MNCN-AI127	*Hyphydrus* sp2	Hyphydrini	EF056686	EF056616	EF056649	EF056574	Madagascar	Andasibe	M. Balke
19	NHM-ER6	*Microdytes* cf. *boukali*	Hyphydrini	EF056694	EF056622	EF056656	EF056584	India	Kallar Valley	D. Boukal
20	MB381	*Microdytes elgae*	Hyphydrini	EF056689	EF056619	EF056652	EF056580	Singapore	Bukit Timah	M. Balke
21	MB457	*Microdytes elgae*	Hyphydrini	EF056692	-	EF056654	EF056583	Myanmar	Alaungdan Kathapa NP	D. Boukal
22	MB456	*Microdytes maculatus*	Hyphydrini	EF056691	EF056620	-	EF056582	Myanmar	Alaungdan Kathapa NP	D. Boukal
23	MB379	*Microdytes pasiricus*	Hyphydrini	EF056690	-	EF056653	EF056581	Singapore	Bukit Timah	M. Balke
24	NHM-IR85	*Microdytes* sp.n.	Hyphydrini	EF056693	EF056621	EF056655	-	Myanmar		H. Schillhammer
25	MB1226*	*Primospes suturalis*	Hyphydrini	EF056698	EF056627	EF056661	EF056589	South Africa	West Cape, Velddrt	G. Challet
26	NHM-IR608	*Allodessus megacephalus*	Bidessini	AY368223	AY368227	EF056630	EF056547	Japan	Honshu, Shizuoka pref.	J. Bergsten
27	NHM-IR95	*Bidessodes mjobergi*	Bidessini	EF056667	EF056595	AJ318725	EF056552	Australia	QLD, Emu	C.H.S.Watts
28	NHM-IR619	*Uvarus peringueyi*	Bidessini	EF056699	EF056628	EF056662	EF056590	South Africa	W Cape, Wilderness NP	I. Ribera & A. Cieslak
29	NHM-IR16	*Yola bicarinata*	Bidessini	EF056700	EF056629	AJ318729	EF056591	Spain	Murcia	I. Ribera
30	NHM-IR616	*Canthyporus parvus*	Hydroporini	EF056668	EF056596	EF056634	EF056553	South Africa	W Cape, Cape of Good Hope	I. Ribera & A.Cieslak
31	NHM-IR40	*Graptodytes flavipes*	Hydroporini	AY250914	EF056604	AJ318730	EF056561	Spain	Huelva	I. Ribera
32	NHM-IR39	*Hydroporus pubescens*	Hydroporini	EF419327	AF309300	AJ318734	EF056566	Spain	Burgos	I. Ribera
33	NHM-IR117	*Laccornellus copelatoides*	Hydroporini	AY334131	AY334247	AJ318738	EF056578	Chile	X Reg., La Unión	I. Ribera
34	NHM-IR46	*Nebrioporus clarki*	Hydroporini	AY250964	EF056623	EF056657	EF056585	Spain	Sevilla	I. Ribera
35	NHM-IR27	*Hydrovatus clypealis*	Hydrovatini	EF056679	EF056609	AJ318716	EF056567	Spain	Girona	I. Ribera
36	NHM-IR136	*Hydrovatus nigrita*	Hydrovatini	EF056680	EF056610	AJ318717	EF056568	Australia	Kakadu	D. Norton
37	MB1227	*Herophydrus inquinatus*	Hygrotini	EF056676	EF056605	EF056641	EF056562	Namibia	Maltahöhe	K. Werner
38	NHM-IR19	*Hygrotus inaequalis*	Hygrotini	EF056681	EF056611	AJ318737	EF056569	Spain	Girona	I. Ribera
39	NHM-IR55	*Laccornis oblongus*	Laccornini	AF309241	AF309298	AJ318715	EF056579	UK	Scotland	D.T. Bilton
40	NHM-IR320	*Celina* sp5	Methlini	EF056669	EF056597	AJ318718/9	EF056554	Brazil	Paraná, Foz do Iguassu	I. Ribera
41	NHM-IR179	*Pachydrus* cf. *politus*	Pachidrini	EF056696	EF056625	EF056659	EF056587	Bolivia	Beni, Carcado	K.B. Miller
42	NHM-IR323	*Derovatellus lentus*	Vatellini	EF056672	EF056600	EF056637	EF056557	Brazil	Paraná, Foz do Iguassu	I. Ribera
43	NHM-IR89	*Australphilus montanus*	Laccophilinae	EF056666	EF056594	AJ318713	EF056551	Australia	NSW, Sth of Woolongong	C.H.S. Watts
44	NHM-IR26	*Laccophilus poecilus*	Laccophilinae	AY334130	AY334246	AJ318714	EF056577	Spain	Girona	I. Ribera
45	NHM-IR648	*Neptosternus hydaticoides*	Laccophilinae	EF056695	EF056624	EF056658	EF056586	Malaisia	Kuala Lipis	M. Balke
46	NHM-IR65	*Philaccolilus bellissimus*	Laccophilinae	EF056697	EF056626	EF056660	EF056588	New Guinea	S Nabire, Cemara River	M. Balke

Appendix S2

Data matrix for the Correspondence Analysis (from Biström *et al.*, 1997 with the addition of *Anginopachria*).

original cha.No.	1	2	3	4	5	6	7	8	9	10	11	12	16	17	19	20	21	22	23	25	26
character No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1 *Andex*	0	0	0	0	0	0	1	0	0	0	0	1	0	0	1	0	0	0	0	0	0
2 *Coelhydrus*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0
3 *Darwinhydrus*	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0
4 *Hydropeplus*	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0
5 *Primospes*	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
6 *Microdytes*	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
7 *Allopachria*1	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0	0	0	0	0	0
8 *Allopachria*2	0	0	0	0	0	0	0	1	0	1	1	0	1	1	0	0	0	0	0	0	0
9 *Allopachria*3	0	0	0	0	0	1	0	1	0	1	1	0	1	1	0	0	0	0	0	0	0
10 *Agnoshydrus*	0	0	0	0	0	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0
11 *Hyphovatus*	0	0	0	0	0	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0	1
12 *Hovahydrus*	0	0	0	0	1	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0
13 *Hyphydrus*1	0	0	0	0	1	0	0	1	0	0	0	0	0	0	1	0	0	0	0	1	0
14 *Hyphydrus*2	0	0	0	0	1	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	1
15 *Hyphydrus*3	0	0	0	0	1	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0
16 *Desmopachria*1	1	1	1	1	1	0	0	1	1	0	0	0	0	0	1	1	0	0	1	0	0
17 *Desmopachria*2	1	1	1	1	1	0	0	1	1	0	0	0	0	0	1	1	1	0	1	0	0
18 *Desmopachria*3	1	1	1	1	1	0	0	1	1	0	0	0	0	0	1	1	1	0	1	1	0
19 *Anginopachria*	1	0	0	0	0	0	0	0	0	0	1	1	0	1	1	0*0	1	0*0	0		

*Not observed, assumed to be the same as other species of the Oriental clade (see Methods).

Appendix S3

List of characters used in the Correspondence Analysis. Numeration follows Biström *et al.* (1997) (characters without variation inside Hyphydrini omitted). See Biström *et al.* (1997) for details and illustrations.

1. Antennomeres 1-2: (0) of about the same width as following segments; (1) wider than following segments.
2. Antennomeres 5-10: (0) cylindrical and of varying length; (1) short and slightly expanded in apical half.
3. Labial palpus: (0) apical pair of sensilla placed close together; (1) apical part of sensilla widely separated.
4. Maxillary palpus: (0) with two or more sensilla; (1) with one apical sensillum.
5. Head, anterior margin: (0) unbeaded and straight; (1) beaded and straight or bend upwards.
6. Head, mediofrontal depression: (0) absent; (1) present.
7. Body, lateral outline: (0) continuous; (1) broken at level of base of pronotum.
8. Body outline: (0) elongate; (1) more or less globular.
9. Pronotum, posterior angle: (0) not produced posteriorad; (1) produced posteriorad.
10. Pronotum, lateral bead: (0) of even width; (1) with posterior dilatation.
11. Prosternal process: (0) with "neck" delimited by a ridge (denticulate in lateral view); (1) with "neck" not delimited by ridge (smooth in ventral view).
12. Prosternal process, apex: (0) reaching metasternum; (1) not reaching metasternum.
16. Elytron, anterolateral angle: (0) not extended anteriorad; (1) extended anteriorad.
17. Elytron, humeral yellow macula: (0) absent; (1) present.
19. Metacoxal process, lateral lobe: (0) covering base of trochanter; (1) reduced, not covering base of trochanter.
20. Metacoxae: (0) not soldered to base of abdomen; (1) soldered to base of abdomen.
21. Metacoxal process, posterior margin of ventral lamina: (0) visible; (1) reduced.
22. Metatibia, longitudinal row of punctures on disc: (0) present; (1) reduced.
23. Metatibia, apical transverse row of spines: (0) continuous; (1) broken medially.
25. Metatibia, longer spur: (0) smooth; (1) serrate.
26. Metatibia, shape: (0) club shaped; (1) cylindrical.