

Multiple ancient origins of neoteny in Lycidae (Coleoptera): consequences for ecology and macroevolution

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Neoteny, the maintenance of larval features in sexually mature adults, is a radical way of generating evolutionary novelty through shifts in relative timing of developmental programmes. While controlled by the environment in facultative neotenic, retention of larval features is obligatory in many species of Lycidae (net-winged beetles). They are studied here as an example of how developmental shifts and ecology interact to produce macroevolutionary impacts. We conducted a phylogenetic analysis of Lycidae based on DNA sequences from nuclear (18S and 28S rRNA) and mitochondrial (*rrnL*, *cox1*, *cob* and *nad5*) genes from a representative set of lineages (73 species), including 17 neotenic taxa. Major changes of basal relationships compared with those implied in the current classification generally supported three independent origins of neotenic in Lycidae. The southeast Asian Lyropaeinae and Ateliinae were in basal positions indicating evolutionary antiquity, also confirmed by molecular clock estimates, unlike the neotropical leptolytines nested within Calopterini and presumably much younger. Neotenic exhibit typical K-selected traits including slow development, large body size, high investment in offspring and low dispersal. This correlated with low species richness and restricted ranges of neotenic lineages compared with their sisters. Yet, these factors did not impede the evolutionary persistence of affected lineages, even without reversals to fully metamorphosed forms, contradicting earlier suggestions of recent evolution from dispersive non-neotenic.

Keywords: obligatory neoteny; evolution; phylogeny; K-strategy; species numbers; net-winged beetles

1. INTRODUCTION

Large phenotypic changes in evolution are rare due to the complexity of developmental programmes, but shifts in the timing of developmental pathways relative to others (heterochrony) are a potential source of evolutionary novelty. Two types of heterochrony can be distinguished (Gould 1977). Adult features as those involved in reproduction may be expressed already in the larval stages and development halts before the organism achieves adult maturity (progenesis). Alternatively, neoteny is a form of heterochrony whereby the expression of larval features may be prolonged while organs for reproduction continue to develop. Hence the adult organism maintains some of its apparently juvenile features (neoteny, ‘remaining young’), e.g. resulting in incomplete metamorphosis and, in extreme cases, the lack of adult stages. These alterations can introduce strongly modified adult phenotypes in an affected lineage whose success and further modifications are subsequently controlled by natural selection (Gould 1977; Alberch 1980; Jablonski 2000; Arthur 2004).

Neotenic modifications frequently are facultative, providing phenotypic plasticity and the potential for alternative strategies under fluctuating conditions (e.g. Gould 1977; Denoel *et al.* 2002; Bonett & Chippindale

2006). However, genetically fixed ontogenetic modifications are found in several groups of beetles (Crowson 1981; Cicero 1988). Neoteny in Coleoptera occurs mainly in the superfamily Elateroidea (click beetles and allies), in particular in soft-bodied groups such as the Lampyridae (fireflies) and Lycidae (net-winged beetles; Crowson 1972, 1981; Lawrence 1982). Reduced sclerotization and a soft flexible abdomen with extensive intersegmental membranes reminiscent of those in the larvae represent a first level of incomplete metamorphic maturation. The reduction of elytra in both sexes and female physogastry causing limited flight ability presumably represents a further degree of this syndrome. Lineages affected by neoteny to the highest degree are found in Lycidae where females lack both pupal and adult stages and retain a larvae-like morphology after the last ecdysis (figure 1; Wong 1996, 1998). These are among the most spectacular beetles, reaching body sizes of 5 cm and more, frequently referred to as ‘trilobite larvae’ due to their appearance.

Neoteny in Lycidae has been reported long ago (Gravely 1915; Mjöberg 1925; Crowson 1972), but only recent work revealed its extent in groups from all major tropical regions (Bocak & Bocakova 1988, 1989, 1990; Miller 1991; Bocak 1995, 2001; Wong 1996; Bocakova & Bocak 1999; Kazantsev 1999, 2002; Bocakova 2005, 2006). However, the evolutionary history of these neotenic remained contentious as morphology-based studies failed to establish their phylogenetic placement.

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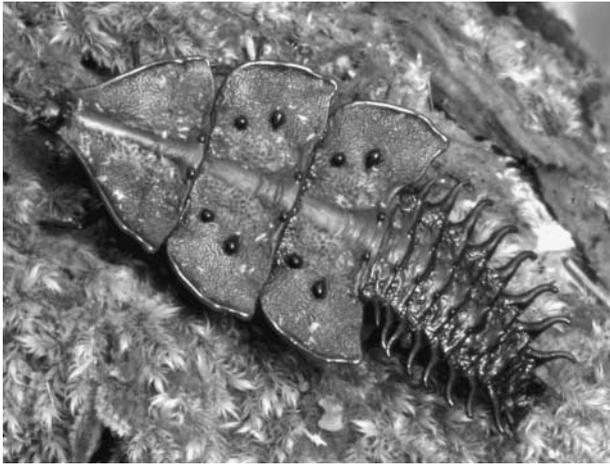


Figure 1. Female larva of *Duliticola* sp. from Mt Sinabung (Indonesia, Sumatra).

Crowson (1972) postulated that neotenus lycids, specifically the southeast Asian genera *Duliticola* and *Lyropaeus*, are members of primitively neotenus lineages that even might include a common ancestor with Lampyridae (fireflies) that equally includes many neotenus forms. The fact that lycids are a widespread cosmopolitan group was ascribed to their secondary return to fully metamorphosed and winged forms from neotenic ancestors. Similar scenarios of evolutionary ‘re-imaginalization’ were proposed by Kazantsev (2005), and equally for the closely related Lampyridae (Cicero 1988). By contrast, Miller (1991) suggested a recent origin of neotenus.

Postembryonic development in these lineages requires several years before maturation (Wong 1996; Bocak & Matsuda 2003), a deceleration of somatic development that is frequently associated with neoteny (Gould 1977). Neoteny is presumably favoured under conditions of slow growth in stable environments that remove the need for a dispersive adult stage and high production of offspring (Gould 1977). The question about early or late origin is critical for assessing the evolutionary persistence and dynamics of diversification in neotenus lineages, and hence to evaluate interactions between development and natural selection. Lycids represent a rare case of genetically fixed neotenus, i.e. they provide a model for studying macroevolutionary consequences of this phenomenon. Neotenic lineages differ from their fully metamorphic ancestors in major life-history parameters, including their low-dispersal propensity resulting from the lack of wings and generally reduced locomotion, and their slow development and hence longer generation time and small offspring numbers. We first ask about the relationships of neotenic groups within Lycidae, to test scenarios of great antiquity and re-evolution of full metamorphosis versus potentially repeated origins of neoteny. Explicit comparisons of sister groups shed light on how neoteny shapes key factors of lineage evolution, including species richness, geographical distribution and range sizes, rarity and the ability of neotenus to react to fluctuating environments.

2. MATERIAL AND METHODS

(a) Sampling, molecular biology methods and data compilation

Sampling of Lycidae included a broad selection of taxa from all zoogeographic regions and representatives of all major

non-neotenic lycid lineages (56 species). The classification of the Lycidae remains unsatisfactory. In the classification of Bocak & Bocakova (1990), the neotenus groups were assigned to four tribes (Dexorini, Leptolycini, Lyropaeini and Ateliini) of which the first three were grouped into the subfamily Leptolycinae. All of these taxa were assigned to subfamily rank by Kazantsev (2005). The phylogenetic analysis described below necessitates major changes to the classification. Although no new names are formally established here, for convenience we use the new taxonomy designated in the electronic supplementary material, table S1. Hence, the following taxa are known to be neotenic: Lyropaeinae from the Oriental region and Sulawesi (Gravely 1915; Mjöberg 1925; Wong 1996; Bocak 2001; Bocakova 2006) represented here by 13 species from 8 (out of 12 known) genera; Ateliinae, an Oriental lineage (Bocak 1995) represented here by *Scarehus*; Leptolycini, a small group from the New World represented in the current study by *Pseudoceratoprion* (Miller 1991); and Dexorinae, known from humid tropical forests of the Afrotropical region (Bocak & Bocakova 1988), which are very rare and were not available for study. Out-groups were five ‘soft-bodied’ elateroid families. A complete list of taxa including geographical origin and GenBank accession numbers are given in the electronic supplementary material, table S2.

All specimens were preserved in 96% alcohol in the field and total DNA was extracted using a phenol/chloroform method (Vogler *et al.* 1993). Three protein coding mitochondrial and three ribosomal genes were amplified. Full-length 18S rRNA (approx. 1900 bp) was amplified as four overlapping fragments (Shull *et al.* 2001). Partial nuclear 28S rDNA, mitochondrial *rrnL*, *cox1*, *cob* and *nad5* genes were amplified as fragments of 420–1220 bp. The primers used for PCR amplifications and conditions used for amplifications are given in the electronic supplementary material, table S3. The total size of the data matrix (under the preferred alignment parameters in CLUSTAL, see below) was 5497 positions; 2459 positions were length-invariable protein-coding mtDNA of which 1643 were parsimony informative versus 242, 107 and 284 informative characters for the length-variable 18S, 28S and *rrnL* markers.

(b) Phylogenetic analysis

Alignments of rRNA and tRNA genes were conducted using CLUSTALX v. 1.8 (Higgins *et al.* 1996) and MAFFT v. 6.502 (Katoh *et al.* 2002) followed by extensive parsimony searches using PAUP* v. 4.0b10 (Swofford 2002). Alignments were performed under a wide range of parameters (table 1 and the electronic supplementary material, table S4). The datasets from all six genes were combined for the final tree search. Equal weights were assigned to all positions and gaps were treated either as missing data or as fifth character state. Overall congruence of the selected alignments with the conserved regions (as a measure of homology and hence to arbitrate between various alignment parameters; Wheeler 1995) was assessed on the incongruence length difference (ILD; Mickevich & Farris 1981), by subtracting the sum of length of the most parsimonious tree of protein coding mtDNA (*cox1*, *cob* and *nad5*; L_x), and variable 18S + 28S + *rrnL* regions (L_y) from the length of trees of combined analysis (L_{x+y}). These ILD measures were normalized according to the length of the combined analysis tree to obtain ILD index values. The congruence of a given topology was also evaluated against the presence of clades previously defined based on morphology (table 1 and the electronic

Table 1. Effect of alignment on tree topology. (Two-step protocols were conducted using CLUSTAL and MAFFT followed by parsimony search under equal weighting (gaps coded as fifth character state) and direct optimization using POY, under a range of gap-opening and extension costs (gap opening : gap extension : nucleotide change for POY). The number of origins of neotenic lineages and monophyly scores of key lineages were obtained under various alignment procedures and parameter settings. The 'score' was based on a count of 1 for monophyletic groups and 0.5 for paraphyletic groups for 10 key taxa. M, monophyletic; P, paraphyletic; —, group absent; Lib, Libnetinae; Lyr, Lyropaeinae; Dic, Dictyopterinae; Sca, Ateliinae; Lyc, Lycinae. A more extensive analysis including further alignment parameters and specific monophyly score for all 10 focal groups is provided in the electronic supplementary material, table S4.)

open/ext	length	ILD	or/rev	Lib	Lyr	Dic	Sca	Lyc	score
<i>CLUSTAL</i>									
2/1	30 435	0.00624	3	M	M	M	M	M	10
2/0.1	30 538	0.00671	3	M	M	—	M	M	8.5
5/1	30 471	0.00704	3	M	M	M	M	M	10
5/0.1	30 444	0.00568	3	M	M	P	M	M	9.0
10/6.66	30 182	0.00918	3	M	M	M	M	M	9.5
10/0.1	30 372	0.00380	3	M	M	P	M	M	9.0
15/6.66	30 039	0.00336	3	M	M	M	M	M	10
20/6.66	30 167	0.00348	3	M	M	P	M	M	9.5
25/6.66	30 507	0.00439	3	M	M	P	M	M	9.0
30/6.66	30 597	0.00536	4	M	—	—	—	P	7.5
45/6.66	30 624	0.00519	3	M	M	M	M	M	10
55/6.66	30 719	0.00531	3	M	M	P	M	M	9.5
75/6.66	30 850	0.00570	3	M	M	P	—	P	7.5
100/6.66	31 051	0.00734	5	M	—	P	—	P	5.0
<i>MAFFT</i>									
LNIS-1	30 285	0.00432	3	M	M	—	M	M	9.0
LNIS-1.5	30 334	0.00498	3	M	M	—	M	M	8.0
LNIS-2	30 330	0.00458	3, 4	M	P	P	—	P	6.5
<i>POY</i>									
1 : 1 : 1	29 683	0.00286	3	M	M	—	M	M	9.0
2 : 1 : 1	29 689	0.00310	3	M	M	M	M	M	8.0
3 : 1 : 1	29 787	0.00356	4	M	M	—	P	M	6.5
3 : 2 : 1	29 776	0.00319	3	M	M	M	M	M	10
4 : 1 : 1	29 848	0.00285	3	M	M	M	M	M	8.0
8 : 1 : 1	30 251	0.00569	3	M	M	—	—	—	6.0
8 : 4 : 1	30 446	0.00670	3/1	M	—	—	—	P	4.5
8 : 8 : 1	30 679	0.00958	2	M	P	P	—	—	3.5

supplementary material, table S4). Nodal support was established by bootstrap analysis generating 100 pseudo-replicates and performing 100 random taxon addition searches each. Topological robustness was also assessed with Bremer support and partitioned Bremer support (Baker & DeSalle 1997), calculated on constraint trees produced with TREEROT (Sorenson 1999). The costs of alternative hypotheses of neoteny origin were computed by constrained tree searches on the matrix produced under default CLUSTALX settings and evaluated with the Shimodaira–Hasegawa (SH) test (Shimodaira & Hasegawa 1999).

Alignment variable regions were also analysed under direct optimization (Wheeler 1996) on the combined six gene partitions using POY v. 3.0.11 (Wheeler *et al.* 2002). Under this procedure, primary homology statements are changeable and chosen based on their lowest cost under parsimony (Wheeler 1996), in contrast to the fixed base-to-base correspondence in the classical two-step approach. The search strategy was based on that of Giannini & Simmons (2003) and consisted of 25 iterations of random addition sequences, with nucleotide transformations minimized with a cost ratio of indels, transversions and transitions. All tree searches involved an initial step of branch swapping, followed by tree fusing (Goloboff 1999) with up to 10 000 fusings allowed and up to 1000 tree fusing trees kept and exchange of subtrees of minimal size during fusing. Tree searches were

performed on a 14 dual-processor (2.8 GHz P4, 2 GB RAM) cluster at Imperial College, London.

To pinpoint shifts in species richness, numbers of known species of all sister clades were compared using the Slowinski & Guyer (1993) measure of tree imbalance. The possible 'trickle-down' effect of species-rich or species-poor nested clades in this analysis was corrected using a heuristic approach (Davies *et al.* 2004). One taxon was selected to represent each clade at the generic, tribal or subfamily level, retaining a total of 38 taxa in the analysis.

Relative age of nodes was estimated using penalized likelihood and cross-validation analysis as implemented in r8s v. 1.71 (Sanderson 2002). Branch lengths were optimized on the preferred tree topology using the GTR+I+G model selected by MODELTEST v. 3.7 (Posada & Crandall 1998). A wide range of smoothing parameters was tested before final analysis. Sampling intervals for inferred divergences were obtained by reanalysing 100 bootstrap replicates of the complete dataset as described in the r8s manual. The age of the in-group was arbitrarily set to 100.

3. RESULTS

(a) Alignment parameters and tree topology

Among a wide range of alignment parameters tested, the ILD values showed the highest congruence of length variable rRNA and protein coding mtDNA in alignments

created by CLUSTAL under gap-opening and gap-extension penalty of 15:6.66 (20:6.66 when treating gaps as missing) and for MAFFT alignments under gap-opening penalty 1.0 and offset value 0.14 (table 1 and the electronic supplementary material, table S4). The resulting topologies (figure 2 and the electronic supplementary material, figure S1) were similar. Most of the signal came from mitochondrial markers (*nad5*, 32.36% and *rrnL*, 21.57%) compared with only 1.81% from 28S rDNA (electronic supplementary material, table S5). The support levels were generally high, but less so for basal branches (figure 2).

The topology agrees only partly with groups defined previously by morphological characters, requiring major changes in the existing taxon concepts (electronic supplementary material, table S1), but phylogenetic conclusions hold up under a wide range of alignment parameters (table 1 and the electronic supplementary material, table S4). The monophyly of Lycidae was highly supported and they can broadly be subdivided in three major lineages: Libnetinae as the sister group of all remaining Lycidae; a clade of Lyropaeinae+Dictyopterinae; and a clade of Ateliinae+Lycinae (figure 2). The clades classified as subfamilies were found in most analyses (table 1). Among these, the Dictyopterinae had the lowest support and under some alignment conditions was either polyphyletic together with *Lycoprogethes* as the sister group of the remaining Lycidae or paraphyletic with Libnetinae included in a basal clade (table 1 and the electronic supplementary material, table S4). However, the latter position is contradicted strongly by morphological traits.

Direct optimization using POY yielded similar trees as the two-step analysis at lower gap costs, while high gap costs produced tree topologies in conflict with morphology, when even the monophyly of genera was not supported (table 1). In the preferred POY trees (based on topological congruence; Wheeler 1995), Libnetinae were placed as sister of the remaining Lycidae under three parameter settings and the sister relationships of neotenic lineages was found exactly as in the two-step analysis (electronic supplementary material, figure S2). Uncertainty affected similar areas of the tree, including the position of the neotenic Lyropaeinae relative to the Dictyopterinae, as under some parameter settings the latter were polyphyletic and Lyropaeinae grouped only with *Lycoprogethes*. Equally, the position of *Pseudoceratoprion* was less stable than that in the two-step protocol.

(b) Multiple origin of neoteny

In the preferred tree, three neotenic lineages corresponding to Lyropaeinae, Leptolycini and Ateliini were widely separated (table 1). This is in contrast to the expectations from the most recent classification scheme of Bocak & Bocakova (1990), which grouped Lyropaeinae and Leptolycini together in the subfamily Leptolycinae (electronic supplementary material, table S1). Three independent origins of neotenic lineages were also encountered under nearly all alignment parameters in CLUSTAL and MAFFT (table 1). Only four topologies suggested four origins when Lyropaeinae were paraphyletic or polyphyletic and in one case five origins were encountered under extreme settings (electronic supplementary material, figure S4). These topologies are rejected on the basis of higher ILD values and owing to

disagreement with morphology-based groups. The Lyropaeinae were the sister of the fully metamorphic subfamily Dictyopterinae (figure 2). The second lineage of southeast Asian neotenic, Ateliinae, was sister to the fully metamorphic tribe Diplophotini in most analyses. The third neotenic group, the neotropical tribe Leptolycini represented by *Pseudoceratoprion*, was consistently found embedded within neotropical Lycini in a terminal position as sister to *Calopteron* (table 1).

Three origins of neotenic lineages were also obtained under 11 of 13 parameter settings in POY. Four origins were obtained under a single setting, splitting Lyropaeinae into two neotenic lineages. Two origins of neoteny were encountered under the extremely high gap costs when *Scarelus* was part of Lyropaeinae, but under these settings even the monophyly of *Scarelus* itself was compromised. On one occasion a reversal was proposed by another extreme setting, but was rejected because it did not recover many expected clades (table 1). Reversal from neoteny to fully metamorphosed females therefore is an unlikely scenario.

When tree searches on the preferred CLUSTAL alignment were constrained for the monophyly of neotenic, tree length increased by 41–55 steps (74–96 steps with gaps coded as fifth character state) for monophyly of all neotenic, or 19–36 (43–61 steps with gaps = fifth character state) if two neotenic lineages were constrained as monophyletic, although SH tests were not significant. Nevertheless, these analyses support the evidence for three independent neotenic lineages.

Extremely large-bodied females, which at present are known only in the southeast Asian subfamily Lyropaeinae, were phylogenetically distant (*Lyropaeus*, *Macrolibnetis* and *Platerodrilus*, figure 2) in most analyses based on CLUSTAL and MAFFT alignments, i.e. they result from multiple origins within this neotenic lineage. In several POY analyses, the tree topology and character optimizations were less clear and involved reversals, but these were usually obtained with extreme settings and the unlikely polyphyly of Lyropaeinae. We expect that females in the other neotenic lineages will be small bodied throughout, although knowledge about these groups is limited. To date, small neotenic females comparable in size to males were described by Miller (1991) for *Leptolycus heterocornis* as a representative of the neotropical neotenic Leptolycini (but not available for molecular analysis).

(c) Species richness and age of neotenic lineages

The neotenic lineages were generally species poor even when not yet formally described taxa deposited in collections are included: neotenic represent 78 known species for lyropaeines, 25 species for ateliines and 40 species for leptolycines, while their respective sister taxa included dictyopterines (120 species), dilophotines (75 species) and *Calopteron* (250 species). Yet, when shifts in species richness were calculated using the algorithm of Slowinski & Guyer (1993), neither of these comparisons was significantly imbalanced. Only three nodes throughout the tree were identified as exhibiting significantly unequal species numbers, including the split between *Libnetis* and all others ($p=0.043$), between Ateliinae and Lycinae ($p=0.024$) and between *Dihammatus* and its sister clade corresponding to all remaining Lycinae ($p=0.024$). With the algorithm to correct for the possibility that a nested

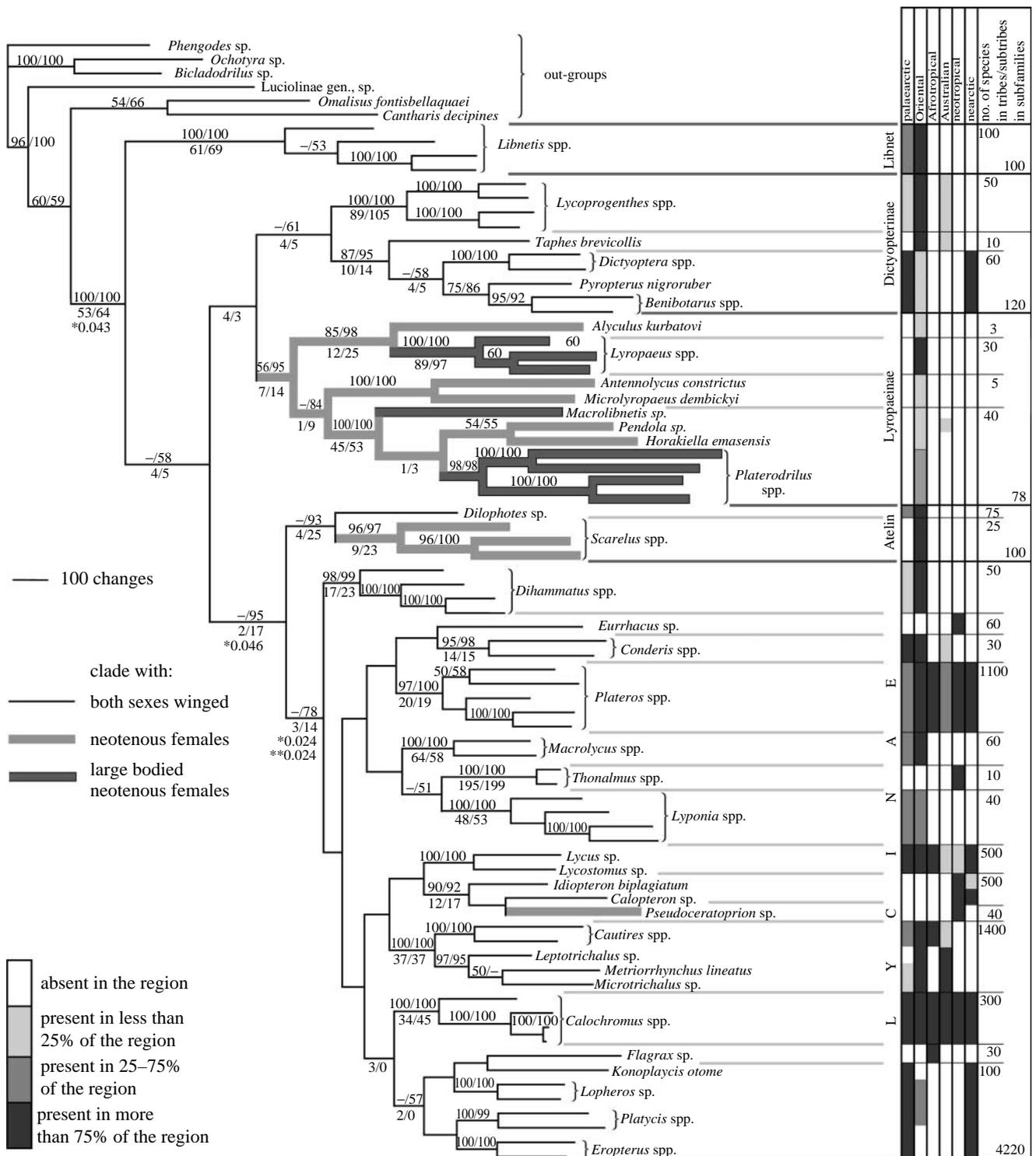


Figure 2. Phylogenetic hypothesis of 73 lycid taxa and six out-groups. One of five most parsimonious trees produced under default settings from the CLUSTALX alignment, analysed with indels considered as missing characters. Numbers above branches refer to bootstrap proportions (if more than 50%) and Bremer support values are given below selected branches. The tree topology is identical with a single tree produced by parsimony analysis of the same dataset when gaps were coded as fifth character. Vertical bars represent the biogeographic distribution of lineages, with species numbers given at the far right. The species numbers are estimations including not yet described species if these are present in collections. Significantly imbalanced nodes: **p* values (Slowinski & Guyer 1993), corrected ***p* values (Davies et al. 2004).

clade causes a significant shift at a higher node (Davies et al. 2004), only the split between *Dihammatus* and its sister clade remains significant, *p*=0.024.

The ranges of all neotenic lineages are restricted to a single zoogeographic region (figure 2) or a part thereof, and no known species is widely distributed. Typically, each species is known from a single mountain range. Their

sister lineages are much more widely distributed as a rule (figure 2).

The relative age estimation of selected clades shows that at least two neotenic lineages, Lyropaeinae and *Scarelus* are ancient and originated among the most basal lineages of the family (at 89.3–95.1 and 62.0–73.7 units, respectively, when the origin of Lycidae was set to 100; the

electronic supplementary material, table S6 and figure S8). The third neotenic lineage split from the fully metamorphic sister group more recently (54.0–65.4 units), but the origin of neoteny cannot be dated with greater precision along the terminal branch, which is defined by a single representative only. Fossils for calibrating the Lycidae are not available, but molecular clock estimates date the diversification of modern elateroid lineages including Lycidae to over 140 Myr BP (Hunt *et al.* 2007). This date is consistent with a time frame inferred for the Lycidae if the split between vicariant lineages of Lycini from South America and Africa is set to 90 Myr BP as the latest time when dispersal over a widening south Atlantic was possible, placing the origin of the two southeast Asian neotenic lineages well into the Cretaceous. The age of lineages with large-bodied females are much younger than the neotenic genera themselves according to this analysis (electronic supplementary material, table S4), consistent with a stepwise evolution of extreme neotenic phenotypes.

4. DISCUSSION

(a) *Origin of neoteny in Lycidae*

Heterochrony is a major source of evolutionary novelty and saltational changes such as metamorphosis in insects provide the raw material for dramatic morphological differences. Since Gould's (1977) seminal book, studies have raised the question about how developmental shifts would affect life histories and, ultimately, macroevolutionary patterns. The comparatively small genetic changes leading to neoteny, presumably affecting the endocrine system, yield very significant shifts in the phenotype (Nijhout 1994). This has inevitable consequences at the level of individuals, which indirectly shape large-scale patterns (Gould 1977; Jablonski 2000). Neotenic beetles have received surprisingly little attention in this context but they are among a small number of cases that can be investigated for the macroevolutionary consequences of these developmental shifts.

Neoteny is presumably favoured under conditions of slow growth in stable environments that remove the need for a dispersive adult stage and for high production of offspring (Gould 1977). In most lineages, as for example in many salamanders, neoteny is reversible and full metamorphosis returns under conditions of environmental stress, providing an escape strategy that ensures the persistence of a lineage. Obligatory neotenic face a high risk of extinction and presumably are evolutionarily short lived. However, several traits have led to suggestions that neotenic Lycidae in fact are ancient, a conclusion that was probably reinforced by the primitive traits evident in larval stages 'recapitulating' the phylogeny of Coleoptera and pterygote insects (Kazantsev 2005), but are most certainly a mistaken interpretation of character homology (Beutel *et al.* 2007). Others have proposed that neoteny itself is reversible (Crowson 1972; Cicero 1988; Kazantsev 2005) as a prerequisite for lineage persistence or that neoteny is recent altogether (Miller 1991).

Our phylogenetic analysis clarifies some of these issues without relying on the possibly misleading (difficult to homologize) and incomplete (unknown larvae and females) information from morphological characters. The extensive phylogenetic analysis based on six gene fragments and

thorough alignment procedures (table 1) provided well-supported trees whose principal results hold up under a wide range of methods for alignment and tree building. The main conclusions are as follows. (i) Two neotenic lineages from tropical Asia (Lyropaeinae and Ateliini) are phylogenetically widely separated and represent ancient groups with a likely origin during the early diversification of lycid lineages in the Mesozoic. A South American lineage of neotenic (Leptolycini) split from the closest fully metamorphosing group later (electronic supplementary material, figure S8), while an African neotenic lineage (Dexorinae) may represent the fourth independent origin but was not available for analysis. (ii) Trait mapping is consistent with the absence of reversals in neoteny, i.e. neotenic groups are not likely to represent a short-lived state in a lineage of mostly fully metamorphosed taxa. The proposed re-imaginalization (Cicero 1988) hypothesized for the morphological changes in related neotenic Lampyridae, Drilidae and Phengodidae would probably not be corroborated either. These groups may further illustrate the ease with which neotenic traits originate in this group of elateroid beetles, while they also show a greater range of morphological modifications (degree of neoteny) including the retention of vestigial wings.

(b) *The evolutionary consequences*

Although neoteny is a well-established phenomenon in lycid beetles, only recently has information become available to address questions about the evolutionary fate and ecological diversity of these lineages. Neoteny is predicted to be adaptive under a K-selection regime, which is evident to an extreme degree in Lycidae. First, the loss of flight in neotenic females reduces the capabilities to disperse and colonize new habitats. Females oviposit immediately after copulation (Wong 1996) in the closest vicinity, and larval dispersal and access to novel resources are also limited. Consequently, most neotenic species are known from a single mountain range or a small continuous part of lowland area only, showing high turnover between areas. This also translates into restricted ranges at the clade level when comparing ranges of fully metamorphosed and neotenic sister lineages (figure 2). Small species ranges, especially on mountain ridges, are island like and may constitute a considerable risk to survival in case of rapid environmental changes. The altitude shifts of forest ecosystems and cyclical aridity during glacial maxima can considerably change the size of such island habitats (Ray & Adams 2001). It is striking that all neotenic lycids included in this study occur exclusively in humid tropics, including the highly stable southeast Asian rainforests (Heaney 1991). Similarly, the Dexorinae is confined to areas of ancient Afrotropical rainforests (Sierra Leone, Cameroon, Gabon, Bioko Island, mountain ranges in Kenya and Uganda; Tallis 1991).

Increase of body size and greater investment in offspring production, also predicted for neotenic (Gould 1977), are clearly evident in Lycidae. The neotenic lineages profit from reduced energetic requirements through the loss of wings and incomplete metamorphosis, which are a trade-off for investment in reproduction (Wagner & Lieberr 1992; Roff 1994; Denoel *et al.* 2002). These trends apparently have occurred in a stepwise fashion evident from basal neotenic such as *Alyculus*, *Microlyropaeus* or *Antennolycus*,

whose females are thought to be comparable in size to males, as is the case in *Leptolycus* (Miller 1991). The most extreme body size in females of the lyropaeine lineage, inferred to have arisen on three occasions (figure 2), is a derived state, which evolved in *Lyropaeus* (26.1–33.2 relative units ago), *Platerodrilus* (43.2–51.0 units ago) and *Macrolibnetis* (after split from their sister group, 58.6–65.1 units ago), presumably as an adaptive response whereby investment in reproduction is channelled towards extremely large eggs (Wong 1996). These trends are not as clear in the males that always undergo full metamorphosis, but in comparison with non-neotenic lycids they are even less active than is usual in this family (only found in the lowest strata of vegetation and in leaf litter). Additionally, all known lycids with reduced elytra in males are members of strongly affected neotenic lineages.

Decreased locomotion is a trait common to all soft-bodied elateroid families. They are incapable of swift running and their flight is slow and reluctant. As an alternative to effective escape mechanisms they achieve protection through poisonous and repellent substances (Moore & Brown 1981), and they are aposematically coloured (Cantharidae and Lycidae; Linsley *et al.* 1961) or use luminescence as an aposematic signal (Lampyridae and Phengodidae; De Cock & Matthysen 2003). Most neotenic females and their larvae live cryptically and are also cryptically coloured. Only when ready for copulation they expose themselves for a short time in prominent places to attract males, while other species live on tree trunks (*Macrolibnetis*) and sometimes forming aggregations (*Platerodrilus* sp., in Crocker Range, Sabah) where they are aposematic. However, none of these defensive strategies are unique to neotenic, as similar colour patterns are known in several non-neotenic lineages, e.g. in Afrotropical and Asian Lycini (Bocak & Matsuda 2003). The origin of these strategies for predator evasion clearly preceded the shift to extreme neoteny. Together with slow locomotion and soft-bodiedness (which may in fact constitute a basic form of neoteny; Bocakova *et al.* 2007), their highly efficient defence mechanism may have provided the evolutionary settings where neoteny became advantageous. It is unlikely that the cause–effect relationship is reversed, i.e. that the habitat selects for low dispersal, as is a common explanation for loss of flight in insects (Wagner & Lieberr 1992; Roff 1994). Those trends are usually associated with extreme environmental conditions and island-like habitats where dispersal probably results in death and is selected against, while the habitat of neotenic is characterized by environmental and geological constancy. Rather than being selected by the environment, flightlessness and low-dispersal ability are *tolerated* in such systems, while the selective regime is for reproductive traits under an extreme K-strategy.

(c) *Speciation in neotenic lineages*

Lycidae provide a possibility to investigate the macro-evolutionary consequences of neoteny. Comparing Lycidae (more than 4000 described species) with their closest soft-bodied relatives (Bocakova *et al.* 2007), the non-neotenic Cantharidae is a large group with some 6000 described species, some 2000 species of Lampyridae of which approximately 25% are neotenic (Cicero 1988). The remaining soft-bodied families of Elateroidea (Bocakova *et al.* 2007) include Phengodidae (glow-worms; 250

species), Rhagophthalmidae (100 species), Drilidae (100 species), Telegeusidae (8 species) and Omalidae (8 species). They are much less species rich and consist almost exclusively of neotenic. Only the Omethidae (40 species) is a non-neotenic species but still species poor. Equally, lycid neotenic represent depauperate clades with some 2% of the species known for this family (figure 2), again supporting the trend of a great reduction in species diversity. Young clade age is unlikely to explain this low diversity, as lyropaeines and ateliines are apparently ancient lineages. It is also unlikely that the low species richness is due to sampling artefacts because neotenic groups have received much attention in field research and publishing activity. In fact, taxonomic efforts lag behind in non-neotenic lineages, as literally thousands of species of Lycidae already available in collections await formal descriptions. Unlike lyropaeines and ateliines, leptolycines are a tip-level group within the species-rich New World clade of Lycini whose evolutionary history started only after separation of South America and Africa (electronic supplementary material, table S4 and figure S8). Although undoubtedly, further leptolycine species have been deposited in collections (M. Ivie 2007, personal communication), the clade is very small compared with its supposed sister group and they are rare in the field.

Possible scenarios for the low diversity in neotenic lineages would benefit from a possibility to separate speciation and extinction rates to study the underlying processes. However, extremely low vagility can cause both the higher frequency of speciation due to the common fragmentation of populations, as well as the decreased speciation rate due to inability to expand ranges and spin off small peripatric populations (Jablonski & Roy 2003). The low species richness, even if not significant in the Slowinski–Guyer test and its modification (figure 2), may therefore be due to the higher vulnerability to extinction in neotenic species resulting from their regularly low abundance and restricted ranges. Yet, despite being at the extinction edge, they were able to persist in the stable environment of tropical forests and gradually acquired more extreme traits related to slow growth and high reproductive investment. These grew out of existing behavioural strategies and existing traits, which apparently favoured the repeated origin of these ontogenetic modifications. Although the female adult morphology is drastically changed in comparison with their ancestors, the apparent ‘novelty’ did not open previously inaccessible adaptive zones, but instead resulted in a refinement of apparent adaptations to an environment that is ecologically stable over geological time-scales. While it is remarkable that these lineages have persisted as long as they did, their life histories clearly limit their long-term existence to climatically constant environmental conditions such as the most ancient rainforests on the planet.

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