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Phylogenetically informative rearrangements in mitochondrial genomes of Coleoptera, and monophyly of aquatic elateriform beetles (Dryopoidea)

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ABSTRACT

Mitochondrial gene order in Coleoptera has been thought to be conservative but a survey of 60 complete or nearly complete genomes revealed a total of seven different gene rearrangements (deletions, gene order reversals), mainly affecting tRNA genes. All of these were found to be limited to a single taxon or a subclade of Coleoptera. The phylogenetic distribution of a translocation of $tRNA^{Pro}$ in three species of elateriform beetles was investigated further by sequencing three nearly complete mitochondrial genomes (Dascillidae, Byrrhidae, Limnichidae) and ten additional individuals for a ~1370 bp diagnostic fragment spanning the relevant region. Phylogenetic analysis consistently recovered the monophyly of families previously grouped in the contentious superfamily Dryopoidea, a group of approximately 10 beetle families with mainly aquatic lifestyles. The Byrrhidae (moss beetles) were not part of this lineage, although they may be its sister group, to recover the widely accepted Byrrhoidea. The $tRNA^{Pro}$ translocation was present in all members of Dryopoidea, but not in any other Elateriformia, providing independent support for this lineage and for a single origin of aquatic habits.

1. Introduction

Gene order rearrangements in the mitochondrial genome have been extremely useful phylogenetic markers (Boore and Brown, 1998). However, their utility seems to be greater in some lineages than others because of differences in the rate at which these rearrangements occur (Dowton et al., 2009). In Coleoptera (beetles) the available sequences suggested a uniform gene order corresponding to the ancestral holometabolan arrangement (Friedrich and Muqim, 2003; Sheffield et al., 2008). This situation differs from other major groups of insects such as the Hymenoptera or Psocoptera that show extensive levels of rearrangements (Dowton et al., 2009; Shao et al., 2003). Yet, the denser taxonomic sampling of mitochondrial genome sequences increases the chances of finding deviations in gene order. Already, inversions of tRNA gene order and a deletion of a tRNA gene are known from recent studies in weevils (Curculionidae) (Song et al., 2010) and the tenebrionoid family Mordellidae (Cameron et al., 2009). Although these conditions each represent a derived character state that is phylogenetically uninformative, they could represent synapomorphies of particular subclades.

Further rearrangements may remain undetected in the mitochondrial genome sequences of Coleoptera, in particular where the relative position of tRNA genes is affected, because phylogenetic analysis of mitogenomes has usually been based on the protein coding regions only that were aligned individually without considering the gene order (Pons et al., 2010; Sheffield et al., 2009; Song et al., 2010; Timmermans et al., 2010). Here, we searched all mitogenome sequences currently available for the Coleoptera for deviations from the presumed panarthropodan (Braband et al., 2010) gene order. This resulted in the discovery of several additional cases of translocations and deletions of tRNA genes.

The phylogenetic distribution of one of these rearrangements, in the superfamily Elateriformia, was investigated in more detail for its taxonomic extent. The Elateriformia is one of the five Series (infraorders) of Polyphaga that includes well-known families such as click beetles (Elateridae), soldier beetles (Cantharidae), jewel beetles (Buprestidae) and glow worms (Rhagophthalmidae, Lampyridae). Basal relationships in the Elateriformia remain uncertain, despite recent progress in particular in the superfamily Elateroidea (Bocakova et al., 2007; Kundrata and Bocak, 2011). Several smaller families have been grouped variously in the superfamilies Byrrhoidea and Dryopoidea whose circumscription conflicts which each other. The Dryopoidea, as defined by Crowson (1981), includes several small families of beetles that are affiliated with aquatic and riparian habitats either in the adult, larval or both stages. However, their

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monophyly has been rejected in all recent cladistic analyses (Beutel, 1995; Costa et al., 1995; Lawrence, 1988; Lawrence et al., 2011), notably through the inclusion of the morphologically divergent family Byrrhidae (moss beetles). Consequently the superfamily Byrrhoidea of Lawrence and Newton (1995) refers to a Dryopoidea expanded by the Byrrhidae, and it was considered closely allied with the Buprestidae (=Buprestoidea). The Byrrhoidea sensu Lawrence and Newton (1995) usually are found to be split into two major groups, sometimes referred to as "Psephenoidea" and "Byrrhoidea" (Costa et al., 1995; Lawrence, 1988). The cladistic analyses performed to date in all cases therefore resulted in the polyphyly of the aquatic lineages. Whether or not the aquatic lineages are monophyletic is important for an understanding of the major transitions in life style of Coleoptera and how these shifts would have contributed to the great species richness of this group.

2. Material and methods

2.1. PCR amplification and sequencing

Nearly complete mitochondrial genomes were newly obtained for species of Limnichidae (Limnichidae gen. sp), Byrrhidae (*Byrrhus* sp.) and Dascillidae (*Dascillus cervinus*), using long-range PCR and 454/Roche sequencing as described previously (Timmermans et al., 2010). Seven additional species (*Cytilus sericeus, Byrrhidae; Limnius volkmari, Elmidae; Oulimnius rivularis*, Elmidae; *Potamodytes* sp., Elmidae; *Chelonarium* sp., Chelonariidae; *Callirhipis* sp., Callirhipidae; *Ptilodactyla serricornis,* Ptilodactylidae), two species of Buprestidae (*Trachys minuta, Julodinae* sp.) and one specimen of Dascillidae (*D. cervinus*) were selected from the NHM frozen DNA collection to target a smaller genomic fragment of approximately ~1370 bp ($tRNA^{Thr}$, $tRNA^{Pro}$, *nad6*, and *cob*) to test the gene order in this particular region. PCR was performed with primer CB4 (located in *cob*; Barraclough et al., 1999) and either primer tRNA-Thr1 5′-AAT ATT GGT CTT GTA AAY C-3′ or tRNA-Thr2 5′-AAC AYT GRT YTT GTA AAY C-3′ (located in $tRNA^{Thr}$) under the following conditions: 3 min at 95 °C; 30 cycles of 30 s at 95 °C, 30 s at 50 °C and 90 s at 72 °C; a final extension of 10 min at 72 °C. Amplicons were purified (Millipore) and bi-directionally sequenced by an in-house sequencing facility using ABI technology. Reads from Sanger sequencing were edited in Sequencher (Genecodes Corp.). All sequences were submitted to GenBank (Accession Numbers JQ034407-JQ034419).

2.2. Gene order

Mitochondrial genome sequences included a set of 25 complete or nearly complete sequences from the Genome database; 28 partially annotated mitochondrial fragments from 454/Roche sequencing (Timmermans et al., 2010); seven sequences generated by Song et al. (2010) newly available on GenBank only since the compilation of Timmermans et al. (2010); and three species newly sequenced in this study. The combined set includes 63 species that represent most major lineages of Coleoptera, including all four suborders and the five Series of Polyphaga, and multiple representatives of some larger families such as Chrysomelidae and Curculionidae (see Timmermans et al., 2010). tRNA sequence prediction was performed with the COVE software package (http://selab.janelia.org/software.html), using Coleoptera specific covariance models generated based on the tRNA annotations of the genomes in GenBank. These models were applied to annotate newly acquired and incompletely annotated sequences, and gene orders were then compared together with the fully annotated mitochondrial genomes available at GenBank using custom-designed BioPerl (http://bioperl.org) scripts.

2.3. Phylogenetic analysis

Sequences of Elateroidea, Buprestoidea and Byrrhoidea were selected from the data matrix of 12 protein coding sequences of Timmermans et al. (2010) and combined with the newly generated sequence data (Table 1). Phylogenetic analyses were performed on

Table 1
Taxa used for the analysis of relationships in Elateriformia.

Superfamily	Family	Species	Voucher (BMNH)	GenBank acc. No.	Sequence	Reference
Buprestoidea	Buprestidae	*Acmaeodera sp.*		NC_013580	PCG	Sheffield et al., 2009
	Buprestidae	*Chrysochroa fulgisissima*		NC_012765	PCG	Hong et al., 2009
	Buprestidae	*Julodinae sp.*	679324	JQ034412	cob–nad6	This study
	Buprestidae	*Trachus minuta*	679281	JQ034411	cob–nad6	This study
Byrrhoidea	Byrrhidae	*Byrrhus sp.*	840448	JQ034419	PCG	This study
	Byrrhidae	*Cytilus sericeus*	833089	JQ034415	cob–nad6	This study
	Callirhipidae	*Callirhipis sp.*	693598	JQ034413	cob–nad6	This study
	Chelonariidae	*Chelonarium sp.*	679389	JQ034409	cob–nad6	This study
	Elmidae	*Elmidae gen. sp.*	840216	HQ232810	PCG	Timmermans et al., 2010
	Elmidae	*Limnius volmari*	679263	JQ034407	cob–nad6	This study
	Elmidae	*Oulimnius rivularis*	679264	JQ034408	cob–nad6	This study
	Elmidae	*Potamodytes sp.*	679360	JQ034410	cob–nad6	This study
	Eulichadidae	*Eulichas sp.*	840452	HQ232812	PCG	Timmermans et al., 2010
	Heteroceridae	*Heterocerus fenestratus*	840449	HQ232811	PCG	Timmermans et al., 2010
	Limnichidae	*Limnichidae gen. sp.*	842697	JQ034416	PCG	This study
	Ptilodactylidae	*Ptilodactyla serricornis*	693606	JQ034418	cob–nad6	This study
Dascilloidea	Dascillidae	*Dascillus cervinus*	679199	JQ034417	cob–nad6	This study
	Dascillidae	*Dascillus cervinus*	833042	JQ034414	PCG	This study
Elateroidea	Cantharidae	*Cantharis pellucida*	840465	HQ232817	PCG	Timmermans et al., 2010
	Cantharidae	*Chauliognathus opacus*		NC_013576	PCG	Sheffield et al., 2009
	Drilidae	*Drilus flavescens*	840459	HQ232815	PCG	Timmermans et al., 2010
	Elateridae	*Pyrophorus divergens*		NC_009964	PCG	Arnoldi et al., 2007
	Eucnemidae	*Melasis buprestoides*	840454	HQ232813	PCG*	Timmermans et al., 2010
	Lampyridae	*Drilaster sp.*	840462	HQ232816	PCG**	Timmermans et al., 2010
	Lampyridae	*Pyrocoelia rufa*		NC_003970	PCG	Bae et al., 2004
	Lycidae	*Merolycus dentipes*	840457	HQ232814	PCG	Timmermans et al., 2010
	Phengodidae	*Rhagophthalmus lufengensis*		NC_010969	PCG	Li et al., 2007
	Phengodidae	*Rhagophthalmus ohbai*		NC_010964	PCG	Li et al., 2007

BMNH, British Museum (Natural History); PCG, all protein coding mitochondrial genes except 5′ *cox1* and *nad2*.
* *cox1* unavailable.
** *nad5*, *nad4*, *nad4L* and *nad6* unavailable.

the *cob* and *nad6* fragments, which avoids missing data, and on the full matrix of 12 protein-coding regions.

Models of sequence evolution best suited to the data were determined with MrAIC (Nylander, 2004) for each of the three codon positions; for first and second codon position combined; and the full dataset. The selected model for each codon position, if analyzed separately or combined was GTR + I + Γ for the data set composed of 12 protein coding genes. Likewise, GTR + I + Γ was selected for the *cob–nad6* fragment, but 1st and 3rd codon positions analyzed separately required the HKY + I + Γ model, and the 2nd codon position required the GTR + Γ model. Tree searches using unpartitioned data were performed in Maximum Likelihood (ML) analyses using Phyml 3.0 (Guindon et al., 2005), with 100 bootstrap replicates to establish nodal support. For Bayesian inferences, for each of the codon positions an independent model was selected with unlinked parameters for the partitions based on the Bayesian Information Criterion (Posada and Buckley, 2004). Searches were conducted in MrBayes version 3.1.2 (Huelsenbeck and Ronquist, 2001), either using all codon positions or under RY-recoding (Hassanin, 2006) of first codon positions and removal of third positions (Pons et al., 2010). In each case, two independent searches were conducted using a single chain (no heating) and the default priors starting with random trees running for 20 million generations sampled at intervals of 1000 generations. Following the MrBayes Manual, heating is frequently not needed for analyses involving less than 50 taxa, which we can confirm from conducting the analysis several times and always receiving the same tree and very similar values for likelihood and posterior probabilities. The use of a single chain greatly speeds up the tree searches. The software programs AWTY (Nylander et al., 2008) and Tracer (Rambaut and Drummond, 2009) were used to assess convergence. The first 10% of trees was discarded as burn-in based on these results, and a majority rule consensus topology from both chains was calculated. Bayes Factors were estimated using Tracer to compare the topologies from the constrained and unconstrained tree searches on the dataset with RY coded first codon positions and removed third codon positions.

3. Results

The survey of published mitogenome sequences revealed several deviations from the ancestral arthropod gene order, particularly in a cluster of six tRNA genes bracketed by the *nad3* and *nad5* genes (Fig. 1). These changes include: (1) the reversal of order of the $tRNA^{Ala}$ and $tRNA^{Arg}$ genes in *Peploptera acromnalis* (Chrysomelidae) and *Naupactus xanthographus* (Curculionidae); (2) an order reversal of $tRNA^{Asn}$ and $tRNA^{Arg}$ in *Mordella atrata* (Mordellidae); (3) an order reversal of $tRNA^{Arg}$ and $tRNA^{Asn}$ – $tRNA^{Ser}$ in *Ischalia* sp. (Anthicidae); (4) a deletion of $tRNA^{Ile}$ in *N. xanthographus* and *Sphenophorus* sp. (Curculionidae); (5) an insertion of nearly 150 bp of extraneous sequence into the (highly divergent) $tRNA^{Ala}$ gene in *Merolycus dentipes* (Lycidae), (6) an order reversal of $tRNA^{Pro}$ and *nad6* in *Eulichas* sp., *Heterocerus fenestratus* and *Elmidae* sp. (Byrrhoidea).

Only two of these rearrangements are potential synapomorphies for more than one of the sequenced taxa, in Curculionidae and Byrrhoidea. Given that representatives of three families of Byrrhoidea showed the $tRNA^{Pro}$ – *nad6* translocation, we investigated if this rearrangement is a synapomorphy for certain subclades within Byrrhoidea. Three nearly complete mitochondrial genomes were obtained and 10 additional representatives of Byrrhoidea covering most established families were sequenced for a ~1370 bp region covering the *cob* to $tRNA^{Thr}$ fragment that covers the relevant region. We found the $tRNA^{Pro}$ – *nad6* translocation to be present in all representatives of Elmidae, Ptilodactilydae, Eulichadidae, Chelonariidae, Callirhipidae, Heteroceridae and Limnichidae, but to be absent from the two representatives of Byrrhidae and all other taxa.

Phylogenetic analysis was conducted under ML and Bayesian methods on the *cob* + *nad6* sequences (no missing data) or on the full matrix of 12 protein-coding regions (with missing data for taxa sequenced for *cob* + *nad6* only). Each of the analyses was performed either on all nucleotide positions; on 1st (RY coded) and 2nd positions only; and under partitioning by nucleotide position (Bayesian analysis only) (Table 2). All analyses recovered three superfamilies of Elateriformia as monophyletic, including the Dascilloidea, Elateroidea (including Cantharoidea) and Buprestoidea, although the elateroid *Melasis buprestoides* (Eucinetidae) was grouped with Buprestoidea in some cases (Table 2). In contrast, the Byrrhoidea *sensu* Lawrence and Newton (1995) was consistently paraphyletic because of the separation of Byrrhidae from the remaining families, while the aquatic families (Dryopoidea of Lawrence and Newton, 1995) were monophyletic. The relationships of these five major lineages differed, in particular the relative

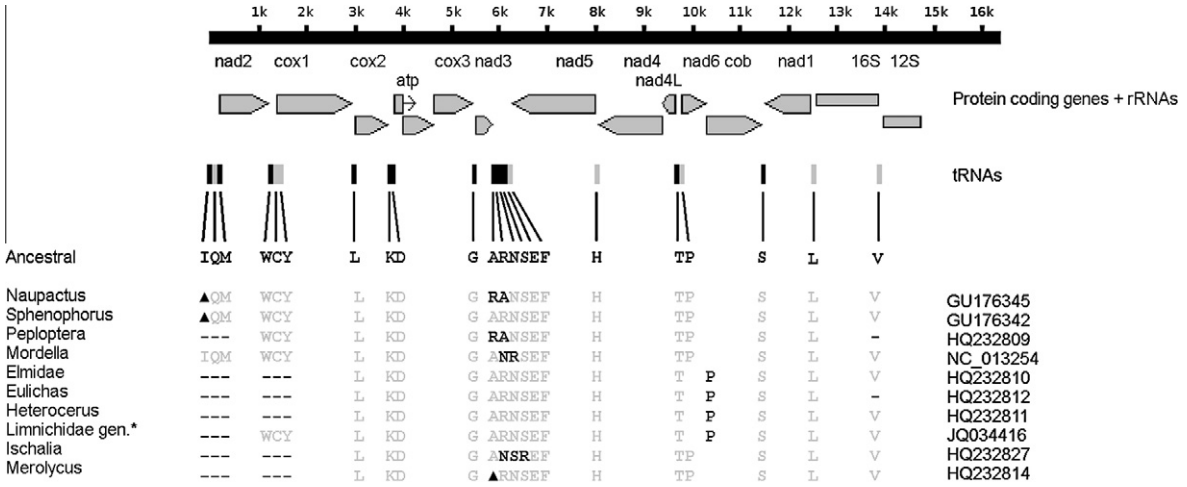

Fig. 1. Gene order in mitochondrial genomes of Coleoptera. The panel at the top shows the map of protein coding, rRNA and tRNA genes representing the ancestral gene order. Arrows pointing to the right (and black bars for tRNA genes) represent genes on the plus strand, arrows pointing to the left (and gray bars for tRNA genes) represent genes on the minus strand. Taxa that deviate in their gene orders are given in the bottom panel. tRNA genes are labeled with a single-letter code. Dashes indicate missing data. ▲ denotes a missing tRNA gene. *, sequenced in the current study.

Table 2
Summary of tree searches.

	Codon positions	Search	No. partitions	Monophyly Byrrhoidea	Monophyly Dryopoidea (BS/PP)	Topology
Cob–nad6	1, 2, 3	ML	–	No	Yes (19)	(Ela(Das(Byr(Dry,Bup*))))
	1RY, 2	ML	–	No	Yes (40)	(Ela(Das(Byr(Dry,Bup*))))
	1, 2, 3	Bayes	3	No	Yes (0.99)	(Ela(Das(Byr(Dry,Bup*))))
	1RY, 2	Bayes	2	No	Yes (0.66)	(Ela(Das(Byr(Dry,Bup*))))
12 genes	1, 2, 3	ML	–	No	Yes (33)	(Ela(Byr(Dry(Bup,Das))))
	1RY, 2	ML	–	No	Yes (100)	(Ela(Bup(Byr(Dry,Das))))
	1, 2, 3	Bayes	3	No	Yes (1.00)	(Ela(Bup(Dry(Byr,Das)))
	1RY, 2	Bayes	2	No	Yes (1.00)	(Ela(Bup(Byr(Dry,Das)))); Fig. 2
	1RY, 2	Bayes	2	Constr.	Yes (1.00)	(Ela(Bup(Das(Byr,Dry)))); Fig. 3

* *Melasis buprestoides* (Elateroidea: Eucnemidae) within Buprestoidea. Ela, Elateroidea; Das, Dascilloidea; Byr, Byrrhidae; Dry, Dryopoidea; Bup, Buprestoidea. 1RY, first codon position RY coded; Constr, constrained for monophyly.

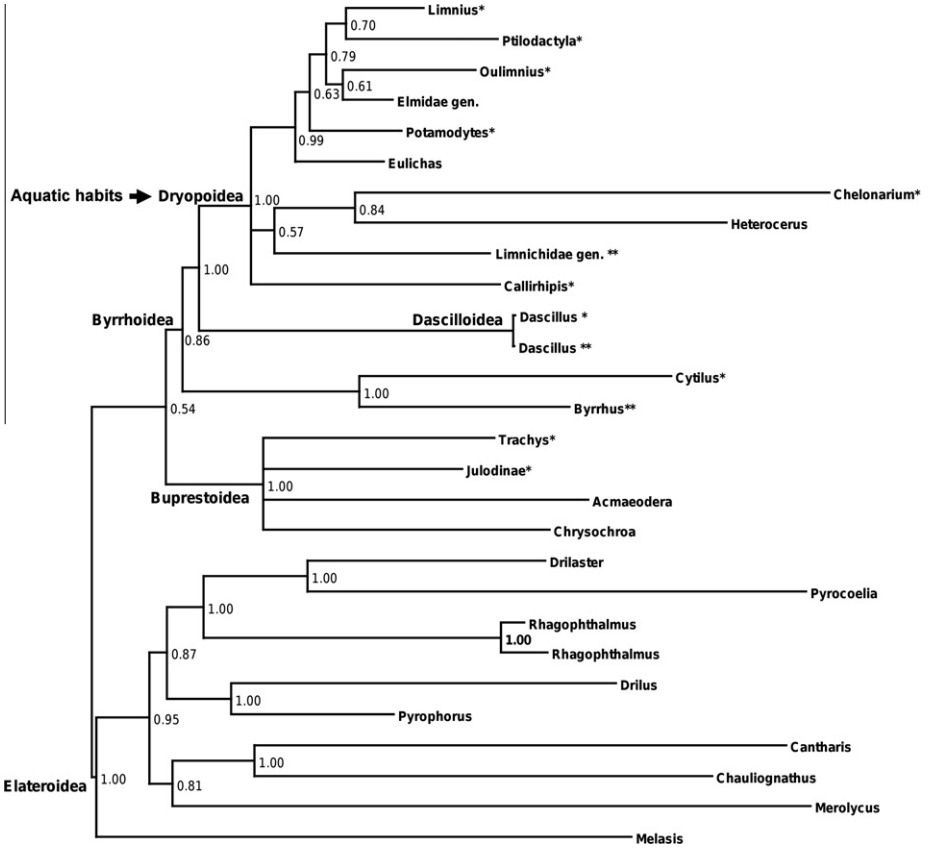

Fig. 2. Relationships among elateriform beetles based on mitogenome sequences. The diagram shows an unrooted Bayesian tree obtained from 12 protein coding genes with first codon positions RY coded and 3rd positions removed. Asterisks mark terminals newly sequenced in the current study (one asterisk, *cob–nad6* fragment sequenced only; two asterisks, nearly complete mitochondrial genome sequences). All others are based on mitochondrial genome data available publicly. Numbers on branches are posterior clade probabilities from the Bayesian analysis.

position of Dryopoidea, Byrrhidae and Buprestidae, which were either placed in a clade (Elateroidea (Dascilloidea (Byrrhidae (Dryopidea, Buprestidae)))) when analyzing the *cob–nad6* fragment only, or included the Dascilloidea for (Elateroidea, Buprestidae (Dryopoidea (Byrrhidae, Dascilloidea)))) when using all data (Fig. 2). However, if Dryopoidea + Byrrhidae were constrained for monophyly, i.e. enforcing a monophyletic Byrrhoidea, the resulting trees were only marginally worse ($\log_{10}$BF = 1.03–1.13) and the Byrrhidae remained the sister to all other families in this lineage (Fig. 3). In all analyses the Dryopoidea of Lawrence and Newton (1995) was highly supported (pp: 1.0), which confirmed the *cob–nad6* translocation as a synapomorphy of the Dryopoidea.

4. Discussion

The mitochondrial genomes of Coleoptera initially have been thought to be largely unchanged from the ancestral gene order (Friedrich and Muqim, 2003; Sheffield et al., 2008), but denser taxon sampling is now revealing several rearrangements that may be phylogenetically informative. In 63 mitogenome sequences available to us, we found a total of seven types of gene orders based on six different translocations or deletions (Fig. 1). These changes affected almost exclusively the tRNA genes, which is expected due to their higher apparent mobility relative to protein coding genes (Moritz et al., 1987). In addition, several mitogenome

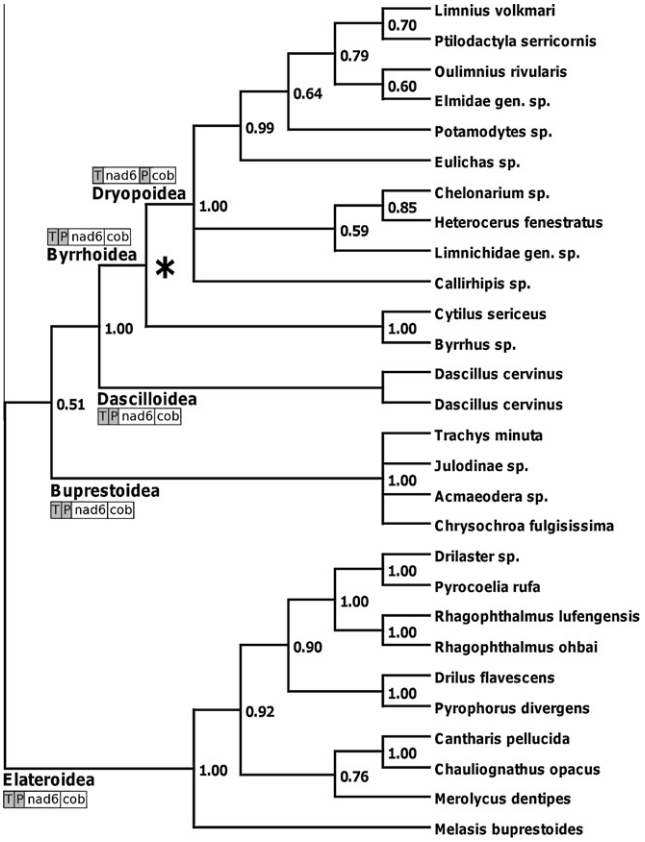

Fig. 3. Bayesian tree from search constrained for a monophyletic Byrrhoidea (*), showing major taxa and the phylogenetic placement of the tRNA^{Pro} translocation.

sequences were incomplete for the control region and the regions around the ribosomal RNA genes and *nad2* genes (Fig. 1), and hence may contain additional deviations from the ancestral gene order not detected here. The changes also included a deletion of a tRNA gene while in another case a disruption of tRNA^{Ala} presumably renders the gene product non-functional. These deletions and insertions are curious, as they suggest gene loss without the presence of functional copies elsewhere in the genome, although they might involve translocations to unsequenced portions (Song et al., 2010). A further rearrangement (an order reversal of tRNA^{Phe} and tRNA^{Glu}) in *Tribolium* (Tenebrionidae) has been reported (Kim et al., 2009), which contradicts the original description of this genome (Friedrich and Muqim, 2003), but after our reanalysis of the sequence this conclusion appears to be based on an incorrect annotation of the tRNA^{Phe} gene.

The utility of mitochondrial gene rearrangements as clade markers was clearly evident in the Dryopoidea. The initial screen of existing mitogenomes revealed multiple taxa with the tRNA^{Pro} – *nad6* translocation, which was further explored for the precise clade limits using PCR amplification of the relevant region. The analysis pointed to a deep origin of this character, in the ancestor of a lineage that has diversified as early as the Jurassic, based on molecular clock analysis of the Coleoptera (Hunt et al., 2007). The tRNA^{Ile} deletion in *Naupactus* and *Sphenophorus* is a further potential synapomorphy for a subgroup of weevils (Curculionidae). This kind of character may help eventually to establish the extent of sub-familial groupings of the Curculionidae that have been notoriously difficult to separate from each other (McKenna et al., 2009). In addition, the tRNA^{Ala} – tRNA^{Arg} change in gene order is shared between *Naupactus* and *Peploptera*, a member of Chrysomelidae (leaf beetles), potentially joining the Phytophaga. However, this represents a case of homoplasy, as each of these species have close relatives (including *Sphenophorus* in the case of *Naupactus*) that exhibit the ancestral gene order. All other rearrangements affect just a single taxon represented in the study, but this does not preclude a wider distribution of these markers, considering that the current taxon sampling is limited to isolated lineages. For example, two different rearrangements were found in *Mordella* and *Ischalia* in the superfamily Tenebrionoidea. They are taxonomically distant from each other and rearrangements in either species may still define larger clades. Similarly, the tRNA^{Ala} – tRNA^{Arg} gene order change in *Peploptera* and *Naupactus*, despite being homoplastic, may well define larger groups, e.g. at the subfamily level in both Chrysomelidae and Curculionidae that remains sparsely sampled.

While not entirely free of homoplasy, at least in the case of Dryopoidea the genomic rearrangement provided an important clade marker. Traditional taxonomists grouped all (semi)aquatic families of Elateriformia as "Dryopoidea" (e.g. Crowson, 1978), but their monophyly subsequently was not confirmed (Beutel, 1995; Lawrence, 1988; Lawrence et al., 2011). The relationships of the dryopoids to the other elateriform superfamilies, Buprestoidea, Dascilloidea, Elateroidea and Cantharoidea have also been difficult to resolve. Affinities of Buprestoidea with dryopoids were first recognized by Crowson, 1982, superseding earlier views that linked them to other hard-bodied groups of the Elateroidea including Elateridae (click beetles) and Eucnemidae in the "Sternoxia". The phylogenetic separation of dryopoids from the Elateroidea (which are now known to include the soft-bodied Cantharoidea) is well supported by morphological (Lawrence and Newton, 1982) and molecular (Bocakova et al., 2007) data. The Dascilloidea also are well established as a basal branch of the Elateriformia but their precise affinities remain unclear (Lawrence and Newton, 1982) and in the current analysis they show strong affinities to Dryopoidea (Fig. 2). In Lawrence & Newton's (1995) widely used classification, the superfamily Byrrhoidea is proposed to include the aquatic families plus Byrrhidae, but it is considered separate from the closely allied Buprestidae (=Buprestoidea). Lawrence et al.'s (2011) recent morphology-based phylogeny of Coleoptera inserts Buprestidae into the dryopoid clade, rendering Dyropoidea polyphyletic. The Dryopoidea were split into two separate clades, separating the Psephenoidea (Psephenidae, Ptilodactylidae) from the remaining Dryopoidea (Elmidae, Heteroceridae, Limnichidae), the latter being sister to Buprestidae and Byrrhidae (Lawrence et al., 2011). Molecular studies to date have been equivocal; whereas a complicated picture was obtained in a study of Elateriformia (mainly focused on other questions in this group) (Bocakova et al., 2007), Hunt et al.'s (2007) tree of Coleoptera finds a monophyletic Dryopoidea, as sister to Buprestoidea and a distantly related Byrrhidae.

The genomic rearrangement now resolves these various possibilities in favor of a monophyletic Dryopoidea, whose sister group are either Byrrhidae or possibly Dascilloidea or Buprestidae (Table 2, Fig. 2). The monophyly of the aquatic families is also supported by the presence of a unique type of wing folding ("dryopoid" type of Forbes, 1926, the loss of functional spiracles on the 8th abdominal segment in adults (with a reversal to functional spiracles in Dryopidae, Heteroceridae and Limnichidae) and the unisetose tarsungulus of the larva. Despite a high degree of ecological diversification, all families in this group are associated with aquatic habitats, ranging from fully aquatic in all stages (e.g. Elmidae), fully aquatic larva and terrestrial adults (e.g. Psephenidae, Ptilodactylidae), fully aquatic adults and terrestrial larvae (some Dryopidae), or both adults and larvae riparian (some Limnichidae, Heteroceridae) (Crowson, 1981; Jäch, 1998). There are also several reversals to a terrestrial lifestyle within fully aquatic groups, usually affecting isolated taxa only. The phylogenetic analysis and the finding of

a clade marker defining the aquatic clade supports an evolutionary scenario in which the aquatic lifestyle originated only once in Elateriformia.

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References

Arnoldi, F.G.C., Ogoh, K., Ohmiya, Y., Viviani, V.R., 2007. Mitochondrial genome sequence of the Brazilian luminescent click beetle *Pyrophorus divergens* (Coleoptera: Elateridae): Mitochondrial genes utility to investigate the evolutionary history of Coleoptera and its bioluminescence. Gene 405, 1–9.

Bae, J.S., Kim, I., Sohn, H.D., Jin, B.R., 2004. The mitochondrial genome of the firefly, *Pyrocoelia rufa*: complete DNA sequence, genome organization, and phylogenetic analysis with other insects. Mol. Phylogenet. Evol. 32, 978–985.

Barraclough, T.G., Hogan, J.E., Vogler, A.P., 1999. Testing whether ecological factors promote cladogenesis in a group of tiger beetles (Coleoptera: Cicindelidae). Proc. Roy. Soc. B 266, 1061–1067.

Beutel, R.G., 1995. Phylogenetic analysis of Elateriformia (Coleoptera: Polyphaga) based on larval characters. J. Zool. Syst. Evol. Res. 33, 145–171.

Bocakova, M., Bocak, L., Hunt, T., Teraväinen, M., Vogler, A.P., 2007. Molecular phylogenetics of Elateriformia (Coleoptera): evolution of bioluminescence and neoteny. Cladistics 23, 477–496.

Boore, J.L., Brown, W.M., 1998. Big trees from little genomes: mitochondrial gene order as a phylogenetic tool. Curr. Opin. Genet. Dev. 8, 668–674.

Braband, A., Cameron, S.L., Podsiadlowski, L., Daniels, S.R., Mayer, G., 2010. The mitochondrial genome of the onychophoran *Opisthopatus cinctipes* (Peripatopsidae) reflects the ancestral mitochondrial gene arrangement of Panarthropoda and Ecdysozoa. Mol. Phylogenet. Evol. 57, 285–292.

Cameron, S.L., Sullivan, J., Song, H.J., Miller, K.B., Whiting, M.F., 2009. A mitochondrial genome phylogeny of the Neuropterida (lace-wings, alderflies and snakeflies) and their relationship to the other holometabolous insect orders. Zool. Scr. 38, 575–590.

Costa, C., Vanin, S.A., Ide, S., 1999. Systematics and bionomics of Cneoglossidae with a cladistic analysis of Byrrhoidea sensu Lawrence & Newton (1995) (Coleoptera, Elateriformia). Arq. Zool. S. Paulo 35, 231–300.

Crowson, R.A., 1978. Problems of phylogenetic relationships in Dryopoidea (Coleoptera). Ent. Germ. 4, 250–257.

Crowson, R.A., 1981. The Biology of Coleoptera. Academic Press, London.

Crowson, R.A., 1982. On the dryopid affinities of Buprestidae. Coleopts. Bull. 36, 22–25.

Dowton, M., Cameron, S.L., Dowavic, J.I., Austin, A.D., Whiting, M.F., 2009. Characterization of 67 mitochondrial tRNA gene rearrangements in the Hymenoptera suggests that mitochondrial tRNA gene position is selectively neutral. Mol. Biol. Evol. 26, 1607–1617.

Forbes, W.T.M., 1926. The wing folding patterns of the Coleoptera. J. N. Y. Entomol. Soc. 34, 42–68.

Friedrich, M., Muqim, N., 2003. Sequence and phylogenetic analysis of the complete mitochondrial genome of the flour beetle *Tribolium castanaeum*. Mol. Phylogenet. Evol. 26, 502–512.

Guindon, S., Lethiec, F., Duroux, P., Gascuel, O., 2005. PHYML online – a web server for fast maximum likelihood-based phylogenetic inference. Nucl. Acids Res. 33, W557–W559.

Hassanin, A., 2006. Phylogeny of Arthropoda inferred from mitochondrial sequences: strategies for limiting the misleading effects of multiple changes in pattern and rates of substitution. Mol. Phylogenet. Evol. 38, 100–116.

Hong, M.Y., Jeong, H.C., Kim, M.J., Jeong, H.U., Lee, S.H., Kim, I., 2009. Complete mitogenome sequence of the jewel beetle, *Chrysochroa fulgidissima* (Coleoptera: Buprestidae). Mitochondrial DNA 20, 46–60.

Huelsenbeck, J.P., Ronquist, F., 2001. MrBayes: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754–755.

Hunt, T., Bergsten, J., Levkanicova, Z., Papadopoulou, A., John, O.S., Wild, R., Hammond, P.M., Ahrens, D., Balke, M., Caterino, M.S., Gomez-Zurita, J., Ribera, I., Barraclough, T.G., Bocakova, M., Bocak, L., Vogler, A.P., 2007. A comprehensive phylogeny of beetles reveals the evolutionary origins of a superradiation. Science 318, 1913–1916.

Jäch, M.A., 1998. Annotated check list of aquatic and riparian/littoral beetle families of the world (Coleoptera). In: Jäch, M.A., Ji, L. (Eds.), Water Beetles of China, vol. II. Zoologisch-Botanische Gesellschaft in Österreich and Wiener Coleopterologenverein, Wien.

Kim, K.G., Hong, M.Y., Kim, M.J., Im, H.H., Kim, M.I., Bae, C.H., Seo, S.J., Lee, S.H., Kim, I., 2009. Complete mitochondrial genome sequence of the yellow-spotted long-horned beetle *Psacothea hilaris* (Coleoptera: Cerambycidae) and phylogenetic analysis among coleopteran insects. Mol. Cells 27, 429–441.

Kundrata, R., Bocak, L., 2011. The phylogeny and limits of Elateridae (Insecta, Coleoptera): is there a common tendency of click beetles to soft-bodiedness and neoteny? Zool. Scr. 40, 364–378.

Lawrence, J.F., 1988. Rhinorrhipidae, a new beetle family from Australia, with comments on the phylogeny of the Elateriformia. Invertebr. Taxon. 2, 1–53.

Lawrence, J.F., Newton, A.F., 1982. Evolution and classification of beetles. Annu. Rev. Ecol. Syst. 13, 261–290.

Lawrence, J.F., Newton, A.F., 1995. Families and subfamilies of Coleoptera (with selected genera, notes, references and data on family-group names). In: Pakaluk, J., Slipinski, S.A. (Eds.), Biology, phylogeny, and classification of Coleoptera. Museum i Instytut Zoologii PAN, Warzawa, pp. 779–1066.

Lawrence, J.F., Slipinski, A., Seago, A.E., Thayer, M.K., Newton, A.F., Marvaldi, A.E., 2011. Phylogeny of the Coleoptera based on morphological characters of adults and larvae. Ann. Zool. 61, 1–217.

Li, X., Ogoh, K., Ohba, N., Liang, X.C., Ohmiya, Y., 2007. Mitochondrial genomes of two luminous beetles, *Rhagophthalmus lufengensis* and *R. ohbai* (Arthropoda, Insecta, Coleoptera). Gene 392, 196–205.

McKenna, D.D., Sequeira, A.S., Marvaldi, A.E., Farrell, B.D., 2009. Temporal lags and overlap in the diversification of weevils and flowering plants. Proc. Natl. Acad. Sci. 106, 7083–7088.

Moritz, C., Dowling, T.E., Brown, W.M., 1987. Evolution of animal mitochondrial-DNA – relevance for population biology and systematics. Annu. Rev. Ecol. Syst. 18, 269–292.

Nylander, J.A.A., 2004. MrAIC.pl. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

Nylander, J.A.A., Wilgenbusch, J.C., Warren, D.L., Swofford, D.L., 2008. AWTY (are we there yet?): a system for graphical exploration of MCMC convergence in Bayesian phylogenetics. Bioinformatics 24, 581–583.

Pons, J., Ribera, I., Bertranpetit, J., Balke, M., 2010. Nucleotide substitution rates for the full set of mitochondrial protein-coding genes in Coleoptera. Mol. Phylogenet. Evol. 56, 796–807.

Posada, D., Buckley, T.R., 2004. Model selection and model averaging in phylogenetics: advantages of Akaike information criterion and Bayesian approaches over likelihood ratio tests. Syst. Biol. 53, 793–808.

Rambaut, A., Drummond, A.J., 2009. Tracer version 1.5.0. <http://beast.bio.ed.ac.uk>.

Shao, R.F., Dowton, M., Murrell, A., Barker, S.C., 2003. Rates of gene rearrangement and nucleotide substitution are correlated in the mitochondrial genomes of insects. Mol. Biol. Evol. 20, 1612–1619.

Sheffield, N.C., Song, H., Cameron, S.L., Whiting, M.F., 2008. A comparative analysis of mitochondrial genomes in Coleoptera (Arthropoda: Insecta) and genome descriptions of six new beetles. Mol. Biol. Evol. 25, 2499–2509.

Sheffield, N.C., Song, H.J., Cameron, S.L., Whiting, M.F., 2009. Nonstationary evolution and compositional heterogeneity in beetle mitochondrial phylogenomics. Syst. Biol. 58, 381–394.

Song, H.J., Sheffield, N.C., Cameron, S.L., Miller, K.B., Whiting, M.F., 2010. When phylogenetic assumptions are violated: base compositional heterogeneity and among-site rate variation in beetle mitochondrial phylogenomics. Syst. Entomol. 35, 429–448.

Timmermans, M.J.T.N., Dodsworth, S., Culverwell, C.L., Bocak, L., Ahrens, D., Littlewood, D.T.J., Pons, J., Vogler, A.P., 2010. Why barcode? High-throughput multiplex sequencing of mitochondrial genomes for molecular systematics. Nucl. Acids Res. 38.