PHYLOGENY OF SELECTED CHOLEVINI TAXA, BASED ON EVOLUTION OF GENITAL CHARACTERS
(COLEOPTERA: CHOLEVIDAE: CHOLEVINAE: CHOLEVINI)

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ABSTRACT

Many members of the Cholevini tribe of carrion beetles occur in northwestern Europe. Usually, they are hard to identify to species level by general morphology alone. Genital morphology gives the best clue. We have researched the evolution of a selection of Cholevini taxa, based on the mtDNA gene COI, and supplemented this with the evolution of male genital characters. COI sequence data was taken from databases GenBank and BOLD. Morphological male genital data was taken from museum specimens and literature. Using both parsimony and maximum likelihood on the sequence data, most likely phylogenies were constructed. Their congruence with morphological male genital data was subsequently checked. It was found that the COI gene by itself can only support species couples being closely related, but fails to confirm deeper evolutionary relations. Also, congruence with morphological genital evolution is only moderately strong according to our data. We give suggestions on how to improve results with future research.

INTRODUCTION

Carrion beetles of the Cholevini tribe within the Cholevinae subfamily, which in turn is located within the Cholevidae family of Coleoptera insects, live largely underground and feed on carrion and other decaying organic matter. Many members of the tribe occur in northwestern Europe, including The Netherlands (Schilthuizen and Vallenduuk, 1998; Lievers, 2011).

Among the species within the Cholevini tribe, very few general morphological characters can be used to discriminate between species (Schilthuizen and Vallenduuk, 1998). For subterranean Coleoptera in general, this has been supposed to be the result of convergent evolution (Decu and Juberthie, 1998 cited in Fresneda, Saldago and Ribera, 2007, p.332), supposedly because these species live under generally similar conditions with equal selection pressures.

In contrast to the moderately disjunctive general evolution of morphological characters shown by Cholevini beetles, evolution of the genital parts can take place at a markedly fast pace. Such has been described by, amongst others, Schilthuizen (2002; 2003; 2007) and Werner and Simmons (2008). Several mechanisms causing this fast evolution have been proposed, with sexual selection by female choice, male-male conflict and male-female conflict likely being the most influential (Schilthuizen, 2003). Major interspecific variation of the genitals within the Cholevidae family was described already long ago by Jeannel (1936) and is nowadays used widely in identification keys for this family (Schilthuizen and Vallenduuk, 1998; Salgado Costas, Blas Esteban, Esteban and Fresneda, 2008).

Our research has focused on determining the evolution of several selected carrion beetle taxa within the Cholevini tribus (genera *Catops*, *Choleva*, *Nargus* and

Sciodrepoides). This was done firstly based on the mtDNA gene COI, from which a phylogeny was constructed. Thereafter congruence with the evolution of the male genitalia was investigated.

MATERIAL AND METHODS

The method followed can be divided into three distinct parts: 1 acquisition of genetic data, 2 acquisition of morphological genital data and 3 data processing and the integration of both data types.

The first part of our research consisted of building a phylogeny of several Cholevini taxa of interest. It was decided to investigate the well-known mtDNA sequence COI, as this sequence has been shown to be useful at species level, such as in DNA barcoding (Herbert, Cywinska, Ball and deWaard, 2003). Two major DNA databases on the web were searched through: GenBank (Benson, et al., 2005) and BOLD (Ratnasingham and Hebert, 2007). COI sequences were downloaded, and if multiple proper COI sequence per taxon were available, all were taken together and a consensus alignment was made using Geneious Pro 5.5.3 software (Drummond, et al., 2011). Then all taxa were aligned with the use of Geneious, saving a 600 base pair DNA sequence for all taxa. Selection of the Cholevini taxa was based on a combination per taxon of COI data being present in one of the databases and specimens being present in our collection of reference, that of NCB Naturalis, Leiden, The Netherlands. This resulted in 13 Cholevini taxa being studied, plus one taxon from the Ptomaphagini tribe, *Ptomaphagus subvillosus*, serving as an outgroup. The complete list is shown in table 1, together with the number of sequences collected per database. For the genus *Nargus*, three species were selected to be studied, though only for *N. velox* COI data was available. The other two species, *N. wilkini* and *N. algericus*, were only studied morphologically.

Table 1 – Cholevini taxa studied and sources used; naming according to Maddison and Maddison (2007) and Zicha (1999-2011). For the use of COI data, the number of sequences used per source are given.

	Sources of COI data with number of sequences used		Sources of morphological genital data as used			
	GenBank	BOLD	SEM	Schilthuizen and Vallenduuk, 1998	Jeannel, 1936	Salgado Costas, et al., 2008
Ptomaphagus subvillosus (Goeze, 1777) (outgroup)	-	5	Y	Y	Y	Y
Catops nigricans (Spence, 1815)	3	-	Y	Y	Y	Y
Catops morio (Fabricius, 1792)	7	-	Y	Y	Y	Y
Catops tristis (Panzer, 1794)	7	-	Y	Y	Y	Y
Catops neglectus Kraats, 1852	7	-	Y	Y	Y	-
Catops picipes (Fabricius, 1792)	1	-	Y	Y	Y	-
Catops kirbii (Spence, 1815)	-	1	Y	Y	Y	Y
Catops fuscus (Panzer, 1794)	-	1	Y	Y	Y	Y
Catops subfuscus Kellner, 1846	-	1	Y	Y	Y	Y
Sciodrepoides watsoni (Spence, 1815)	1	-	Y	Y	Y	Y
Nargus velox (Spence, 1815)	2	-	Y	-	Y	Y
Nargus algericus Portevin, 1903	-	-	Y	-	Y	Y
Nargus wilkini (Erichson, 1837)	-	-	-	-	Y	Y
Choleva elongata (Paykull, 1798)	-	1	Y	-	Y	-

Table 2 - Morphological male genital characters scored, with their character states as distinguished

	Character	0	1	2	3	4
1	Shape of top of aedeagus	Rounded	Pointed	Trapezoid	Pointed to rounded	Split
2	Knob at top of aedeagus	Present	Absent			
3	Strongest curvature of aedeagus	At base	At top	Middle		
4	Paramere length with regard to penis length	< 75%	> 125%	75< >125%		
5	Asymmetry	Present	Absent			
6	Top of parameres	Narrow	Wide			
7	Setae on top of parameres	Perpendicular	In line			
8	Recess on (top of) aedeagus	Absent	Present			
9	Apical ridge on aedeagus	Absent	Present			
10	Base of parameres	Narrow	Wide			
11	Overall shape of aedeagus	Elongated	Spheroidal			

With the aim to find morphological differences in male genital characters between the species, several sources were collected, researched and data put together. Most importantly, museum specimens from the NCB Naturalis collection were collected and examined. The aedeagus of the male genitalia, usually not more than one mm in length, was photographed using a Jeol SEM, type JSM-5400, at Leiden University. Photographs were studied and selected characters scored. Additional information was taken from literature: Jeannel (1936), Schilthuizen and Vallenduuk (1998) and Salgado Costas, Blas Esteban, Esteban and Fresneda (2008). Table 1 provides an overview of the sources of morphological data per taxon studied. Table 2 lists all genital characters and character states scored. An overview of the character states per taxon is presented in Appendix I.

Genetic data, i.e. COI sequences, were used to infer phylogenetic relations among the taxa being studied. This was done firstly by performing an

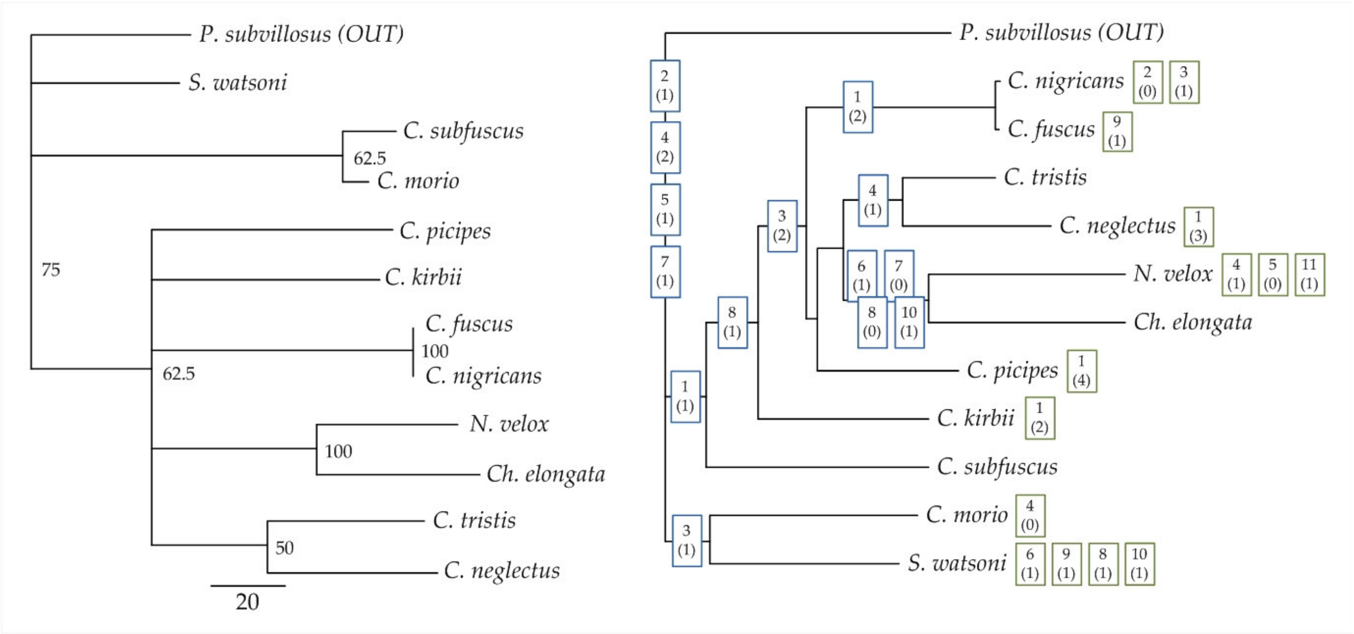

Figure 1 – Phylogenies showing the evolutionary relationships between selected Cholevini taxa. Left as predicted by a 50% consensus of nine most parsimonious trees (with consensus percentages given with all nodes), right as predicted by a maximum likelihood criterion. Plotted on top of the ML tree are the morphological genital characters resulting in synapomorphies (blue boxes) and autapomorfies (green boxes). Per box, upper values show character number, lower values (between brackets) show to what character state this character changes.

exhaustive search in Geneious on the 600 bp DNA sequence, using parsimony as criterion. In order to get a better feeling for relations between the taxa, PAUP was used to perform an heuristic search on the data, now taking maximum likelihood as the criterion. Then the phylogeny as predicted by the maximum likelihood analysis was taken and morphological genital characters were plotted on top to see if and how they corresponded.

RESULTS

The exhaustive search on the COI data resulted in nine evenly most parsimonious trees. The 50% consensus tree is shown in figure 1 on the left side. It is clear that many polytomies arise and the various genera are not located close together, let alone show up as monophyletic.

To gain more insight, a heuristic search was performed on the exact same COI data set. A maximum likelihood (ML) criterion was now used. The result is shown on the right side in figure 1. It shows that sets of two most closely related species turn up in both phylogenies. They are *C. fuscus* – *C. nigricans*, *C. tristis* – *C. neglectus* and *N. velox* – *Ch. elongata*. Other taxa, such as *C. kirbii*, *C. morio*, *C. picipes* and *S. watsoni* are highly inconsistent in their location.

Morphological genital characters as given in Appendix I per taxon are shown in figure 1, as a supplement to the ML phylogeny. Characters were plotted in a most parsimonious way, which resulted in a consistency index (CI) of 0.62. All but two nodes are supported by synapomorphies. Two reversals and two parallelisms were found. Although *C. fuscus* and *C. nigricans* do appear almost similar in both methods, the exact DNA sequences used do show polymorphisms at several base pair locations.

DISCUSSION

Several interesting trends have been found from the phylogenies created with both parsimony and ML as criteria. Most importantly, the 50% consensus phylogeny is highly polytomous. This is a direct result from the nine most parsimonious trees found being very different from each other. Also, we suspect that the COI gene is well suitable when it comes to determining the relationship between two (closely related) species, but fails to reveal the deeper relationships (e.g. between genera as a whole). This idea is further supported by finding several “species couples” with both methods. Performing a bootstrap analysis has been undertaken for both methods, but resulted in too highly polytomous phylogenies to be any informative at all.

The exact relationships between Cholevini beetles seems to be not fully resolved as yet. But, our finding that the species couple *N. velox* – *Ch. elongata* falls within the *Catops* clade seems hardly convincing. The fact that this couple is placed within the *Catops* taxon twice does seem to indicate that this is truly what the COI data shows, though.

We suggest that, to further resolve this issue, at least one other gene should be sequenced and used in building the phylogeny. In order to be more informative, this gene would preferably have to be some less variable at this level than COI. In that way deeper, and thus more ancient, relations could be investigated.

Catops, a large genus with many members in the Palearctic region, has been the subject of relatively many studies. This explains why many COI sequences are provided on GenBank and BOLD for species from this genus. From the other genera, only few sequences are publicly available. The unfortunate consequence for us was that we were left with a skewed distribution of genera in our research, with just one taxon from both *Sciodrepoides*, *Nargus* and *Choleva* each, against eight taxa from the *Catops* genus. Surely a high level of relatedness will then be found between *Catops* taxa, and less between others. Ideally, we would find the other genera outside *Catops*, leaving *Catops* monophyletic. It now appears that the taxa couple *Nargus* – *Choleva* falls within the *Catops* clade. This could well be true, leaving *Catops* a paraphyletic taxon, but should be confirmed by more data. Also more data would be needed to further determine the exact location of placement.

Morphological genital characters are shown in figure 1, as a supplement to the ML phylogeny. Some clades are supported by several synapomorhphies. Good examples are all but the outgroup being a sister group to the outgroup, which confirms our choice for *P. subvillosus* as outgroup species. The species couple *C. nigricans* – *C. fuscus* is also well supported, as is the clade consisting of *N. velox* – *Ch. elongata*.

The CI value for the morphological characters placed on the ML phylogeny of 0.62 appears to be not too bad. Still, quite an amount of homoplasy is present, both in the form of reversals and parallelisms. Character 5, asymmetry, was present in the ancestor, then lost and subsequently regained in all *Nargus* species studied here. The direction of setae on the parameres and the recess found on the aedeagus has been lost and regained multiple times through evolution as well. Parallelisms are an apical ridge on the aedeagus, which originated both in *C. fuscus* and *C. morio*, and a wide base of the parameres, which originated in *C. morio* and the *Nargus* – *Choleva* clade.

Morphologically, all *Nargus* species have been studied. Since no morphological polymorphism within this clade could be detected, and since no COI sequence data was available for two of the three species studied, we can but consider this clade to be rather strongly supported and the three species belonging together indeed (not shown in figure 1).

A general conclusion from current research is that by use of the COI gene, only a moderately accurate phylogeny of the Cholevini carrion beetle family can be constructed. More DNA data would be necessary to increase the resolution above the species level. Moreover, the phylogeny found is only moderately strong supported by morphological genital data.

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APPENDIX I

Data matrix of terminal taxa with character states. Character and character state definitions are given in table 2.

Character number:	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Ptomaphagus subvillosus (Goeze, 1777) (outgroup)	0	0	0	0	0	0	0	0	0	0	0
Catops nigricans (Spence, 1815)	2	0	1	2	1	0	1	1	0	0	0
Catops morio (Fabricius, 1792)	0	1	1	2	1	1	1	1	1	1	0
Catops tristis (Panzer, 1794)	1	1	2	1	1	0	1	1	0	0	0
Catops neglectus Kraats, 1852	3	1	?	1	1	0	1	1	0	0	0
Catops picipes (Fabricius, 1792)	4	1	?	2	1	0	1	1	?	0	0
Catops kirbii (Spence, 1815)	2	1	0	2	1	0	1	1	0	0	0
Catops fuscus (Panzer, 1794)	2	0	2	2	1	0	1	1	1	0	0
Catops subfuscus Kellner, 1846	1	1	0	2	1	0	1	1	0	0	0
Sciodrepoides watsoni (Spence, 1815)	0	1	?	0	1	0	1	0	0	0	0
Nargus velox (Spence, 1815)	?	1	?	1	0	1	0	0	0	1	1
Nargus algericus Portevin, 1903	1	1	?	1	0	1	0	0	0	1	1
Nargus wilkini (Erichson, 1837)	1	1	?	1	0	1	0	0	0	1	1
Choleva elongata (Paykull, 1798)	1	?	?	2	1	1	0	0	0	1	0