

A Taxonomical Comparison of Riverine and Lacustrine *Lamprologus* and Shell Dwelling *Neolamprologus* Species, with Consideration of Morphological and Phylogenetic Classifications

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Abstract

Lamprologini is a tribe of cichlid fish that inhabit Southern Africa. The current taxonomy of the tribe has been called into question by recent phylogenetic studies. Specifically, the classification of *Lamprologus*, a genus which contains both lacustrine and riverine species, has been shown to be inadequate. In order to represent current research and a more realistic portrayal of evolutionary history the splitting of the current genus, separating the riverine species, is suggested. In addition, the inclusion of select shell dwelling *Neolamprologus* species into a group with lacustrine *Lamprologus* species is argued for. This is to better represent current molecular data.

1. Introduction

Recently a great deal of research has been done in the area of cichlid taxonomy, particularly on cichlids that inhabit the African great lakes and surrounding water sheds (Bills and Ribbink 1997; Day et al. 2007; Gashagaza et al. 1995; Market et al. 2010; Salzburger et al. 2002; Sturmbauer et al. 1994; 2010). Cichlids are a group of

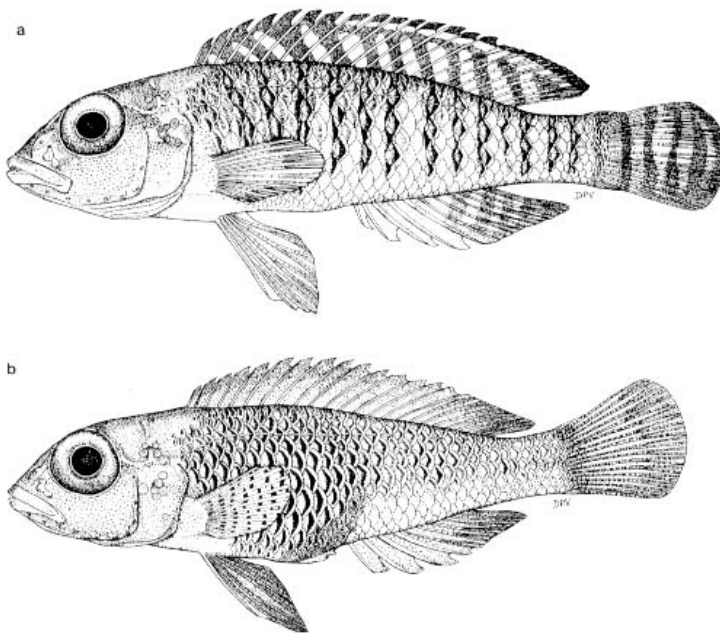


Figure 1: *Lamprologus signatus*, a lacustrine species
a) Male b) Female specimens. (Bills and Ribbink, 1997).

Perciforme, perch-like, fish in the Cichlidae family (Boulenger 1915). A comprehensive investigation of the phylogenetic relationships between cichlid species is still ongoing. The focus of this review is to combine current molecular and morphological data to determine the relationships between the riverine and lacustrine species of the *Lamprologus* genus. Specific shell dwelling species of the genus *Neolamprologus* will also be included to

investigate their relationship to the shell dwellers of the genus *Lamprologus*.

The *Lamprologus* and *Neolamprologus* genera belong to the Lamprologini tribe of cichlid fish, which contains over 90 species in seven genera: *Lamprologus*, *Neolamprologus*, *Altolamprologus*, *Chalinochromis*, *Julido-chromis*, *Lepidiolamprologus*, and *Telmatochromis* (Day et al. 2007). The tribe represents about 40% of the species of Lake Tanganyika (Sturmbauer et al. 2010). The majority of species are endemic to the lake (Figure 1) with the remaining occupying the surrounding river systems (Sturmbauer et al. 2010). In this review 19 species of *Lamprologus* will be considered encompassing 11 lacustrine and 8 riverine. In addition 4 *Neolamprologus* species will be considered and their current classification challenged.

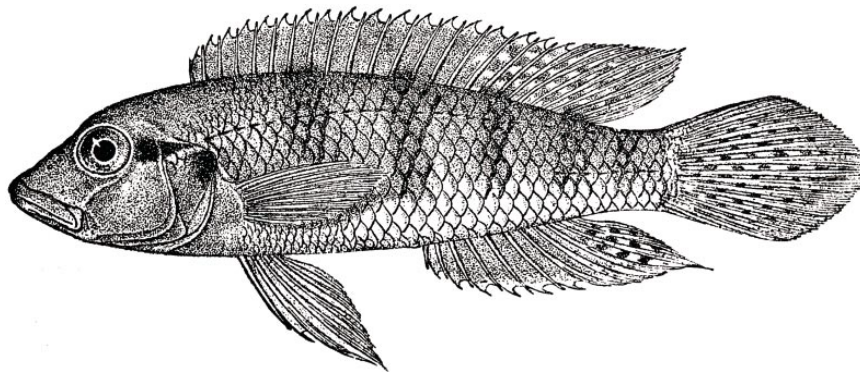


Figure 2: *Lamprologus congoensis*, a riverine species. Male specimen. (Schelly and Stiassny 2004)

The *Lamprologus* genus was originally created by Schilthuis in 1891 and was later broken into the seven genera. The type specimen for *Lamprologus* was *L. congoensis* (Figure 2), originally *L. congolensis* (Boulenger 1915), a riverine species. The last major rework of the Lamprologini tribe was done in 1986 by Max Poll, a taxonomist. Despite evidence of the non-monophyly of *Neolamprologus* the genus has served as a repository for new lamprologine species. This was done anticipating a later revision of the tribe (Day et al. 2007), something that has yet to occur.

Lake Tanganyika is one of the African great lakes, and is the world's second largest lake by volume (Figure 3). It is located between four countries: Tanzania, the Democratic Republic of Congo, Burundi, and Zambia in South Eastern Africa. Lake Tanganyika has very hard alkaline water with a pH of around 9.2. The lake is highly stratified with a well-defined deeper anoxic layer (Konings 2005), which is relatively un-sampled (Day et al. 2007). Lake Tanganyika resembles an inland sea due to its extreme depth, greater than 1,400 meters, and its hard alkaline water. Within the lake there are distinct coastal (littoral) and pelagic zones each supporting their own unique biotope (Konings 2005; Sturmbauer et al. 1994). In addition many groups of marine animals have species that live within the lake including: crabs, jellyfish, and a species of sardine. Lamprologini fish are found predominantly in the littoral zone close to the substrate, specifically over rock, sand,

or mud bottoms (Sturmbauer et al. 1994). A unique habitat of the lake is the shell-beds. These are large stretches of substrate covered completely in gastropod shells of the snail *Neothauma tanganyicense*. These empty shells provide shelter and nesting sites for a variety of small cichlids, including members of the *Lamprologus* and *Neolamprologus* genera (Gashagaza et al. 1995; Sturmbauer et al. 1994). The lake also feeds into the Congo River drainage which eventually empties into the Atlantic Ocean (Konings 2005). The Congo River contains very diverse habitats, with rocky shorelines, mud, flooded grasslands, and deep river canyons (exceeding 200m depth) all being occupied by *Lamprologus* species (Market et al. 2010; Schobert et al. 2012).

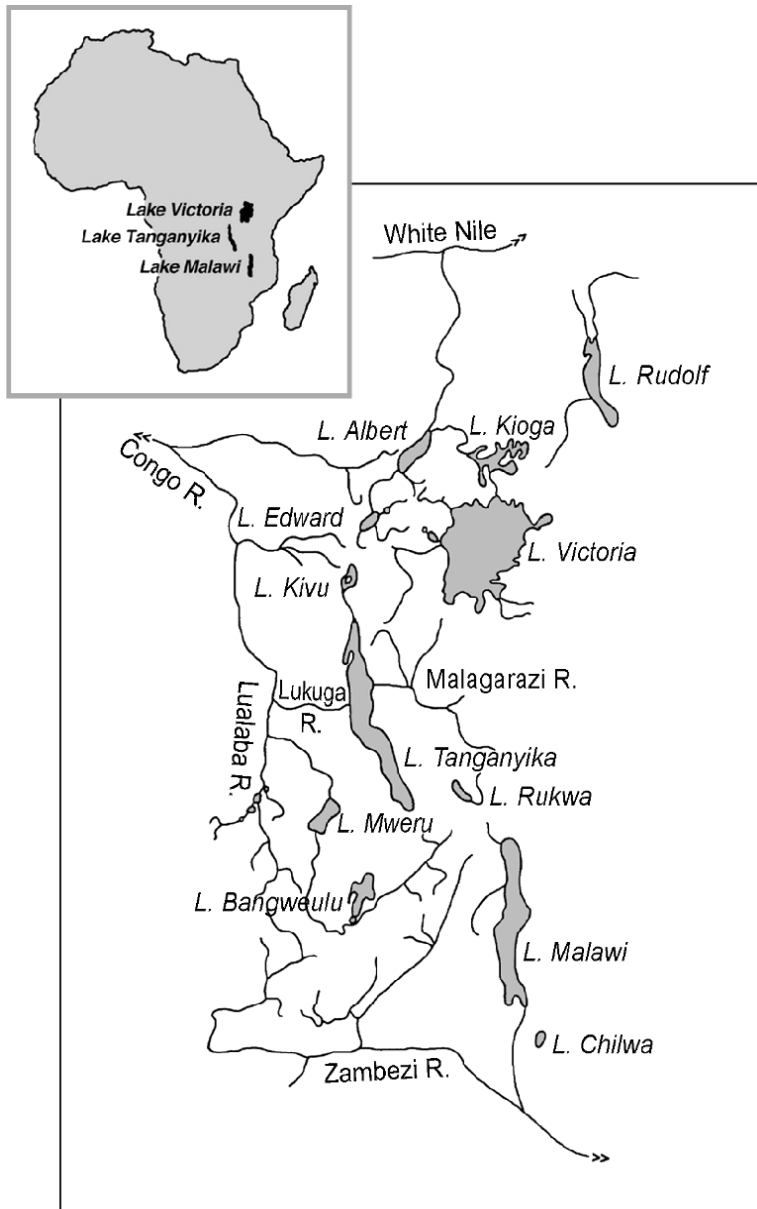


Figure 3: Lakes and Rivers of South East Africa. Lake Tanganyika is connected to the Congo River by the Lukuga River. (Salzburger et al. 2002)

The taxonomy of lamprologine cichlids has been a topic of ongoing research; historically through morphological features and more recently using DNA sequencing (Day et al. 2007; Salzburger et al. 2002; Sturmbauer et al. 1994; 2010). However, no rework of the tribe has resulted from this new information. As stated before, lamprologini, particularly the *Lamprologus* and *Neolamprologus* genera, is recognized as not representing true evolutionary relationships (Day et al. 2007; Sturmbauer et al. 2010). A phylogenetic based organization would more accurately represent these relationships. This has the potential to make the classification of new species more concrete, ensuring that new species are not placed into a repository genus, as is currently being done with *Neolamprologus*. A phylogenetic organization could lead to the assessment of new specimens based purely on muscle or fin samples, allowing for potentially non-lethal sampling. Currently the *Lamprologus* and *Neolamprologus* genera are spread out genetically making it difficult to place new species. This needs to be corrected with current phylogenetic knowledge. Failing to re-evaluate the *Lamprologus* genus will make future research and taxonomy more difficult which would be irresponsible.

Table 1. Species of lamprologine cichlids considered in the review

Species	Habitat	Author
<i>Lamprologus</i>		
<i>L. callipterus</i>	Lacustrine	Boulenger 1906
<i>L. congoensis</i>	Riverine	Schilthuis, 1891
<i>L. finalimus</i>	Lacustrine	Nichols and La Monte 1931
<i>L. kungweensis</i>	Lacustrine	Poll 1956
<i>L. laparogramma</i>	Lacustrine	Bills and Ribbink 1997
<i>L. lemairii</i>	Lacustrine	Boulenger, 1899
<i>L. lethops</i>	Riverine	Roberts and Stewart 1976
<i>L. meleagris</i>	Lacustrine	Büscher 1991
<i>L. mocquardi</i>	Riverine	Pellegrin 1903
<i>L. ocellatus</i>	Lacustrine	Steindachner 1909
<i>L. ornatipinnis</i>	Lacustrine	Poll 1949
<i>L. signatus</i>	Lacustrine	Poll 1952
<i>L. speciosus</i>	Lacustrine	Büscher 1991
<i>L. stappersi</i>	Lacustrine	Pellegrin 1927
<i>L. symoensi</i>	Riverine	Poll 1976
<i>L. teugelsi</i>	Riverine	Schelly and Stiassny 2004
<i>L. tigripictilis</i>	Riverine	Schelly and Stiassny 2004
<i>L. tumbanus</i>	Riverine	Boulenger, 1899
<i>L. wernerii</i>	Riverine	Poll 1959
<i>Neolamprologus</i>		
<i>N. brevis</i>	Lacustrine	Boulenger, 1899
<i>N. calliurus</i>	Lacustrine	Boulenger 1906

<i>N. multifasciatus</i>	Lacustrine	Boulenger 1906
<i>N. similis</i>	Lacustrine	Büscher 1992

The goal of this review is to resolve the interrelations between riverine and lacustrine *Lamprologus* species and the link to shell dwelling *Neolamprologus* species. This will include molecular and phylogenetic analysis, historical and present. Despite the small sample of Lamprologini considered, the resolution of the aforementioned interrelationships will have far reaching effects on the overall taxonomical organization of the tribe. The polyphyletic nature of the *Lamprologus* genus, as suggested by Day et al. (2007) and Sturmbauer et al. (2010), will be supported and the inclusion of the *Neolamprologus* species listed in a group containing shell dwelling *Lamprologus* species will be validated. The division of the *Lamprologus* genus into two genera will also be suggested.

2. Body

2.1 Overview of Methods

Two distinct methods of taxonomical classification were used by the studies reviewed; morphological analysis and molecular/ phylogenetic analysis. Morphological analysis relies on the comparison of physical traits between specimens. From the analysis of physical traits a series of potential evolutionary trees can be created through the use of a character matrix. The most parsimonious tree is usually accepted. This is the tree with the least number of evolutionary changes. Morphological trees are limited in the fact that analogies can potentially be considered homologies, because it is difficult to determine if similar structures arose independently (analogies) or from a shared evolutionary history (homologies). The second method of taxonomical classification is the phylogenetic approach. This is the method used by many of the papers reviewed (Day et al. 2007; Sturmbauer et al. 1994; 2010).

2.2 Phylogenetic Methods

Molecular analysis was performed from samples of fin clippings or white muscle tissue. These samples were acquired from field collections (Day et al. 2007; Sturmbauer et al. 2010), preserved collections (Day et al. 2007; Sturmbauer et al. 1994), and through the aquarium trade (Day et al. 2007; Sturmbauer et al. 2010). All specimens were preserved in ethanol. Selected DNA sequences were amplified for analysis (Day et al. 2007). Comparison between samples was done through sequence alignment of base pairs and data matrixes, similar to the character matrixes used in morphological analysis. Historically only morphological methods were available and because of this the early classification of the genera depended on a few examined specimens. Recent studies have focused on resolving the phylogeny of the Lamprologini tribe using current DNA analysis. Comparisons were done between species by analysing DNA fragments.

2.3 Phylogenetic Studies and Results

Sturmbauer et al. (1994) examined the mitochondrial DNA (mtDNA) from 25 species of lamprologine cichlids including *Lamprologus callipterus*, *L. mocquardii*, *L. congoensis*, *L. weneri*, *Neolamprologus brevis*, and *N. calliurus* which are relevant to this review. In addition, the sequences from outgroup cichlids were included in order to place Lamprologini within a larger context of cichlids. The data collected from this molecular analysis was used to construct a series of phylogenetic trees. The most parsimonious tree was recorded as the result. From this tree (Figure 4) the polyphyletic nature of *Neolamprologus* and *Lamprologus* is evident. All three riverine species of *Lamprologus* included in this study (*L. mocquardii*, *L. congoensis*, and *L. weneri*) were grouped together forming a clade with the *Telmatochromis* and *Julidochromis* species considered in the study. The species *Neolamprologus brevis* and *N. calliurus* were also placed in a clade. The main relevant conclusions drawn from this study are that the Congo River lamprologines form a monophyletic group nested within Lamprologini. This means they likely share a common ancestor which left the lake and populated the river system (Sturmbauer et al. 1994) and are not, as was previously thought, an ancestral lineage.

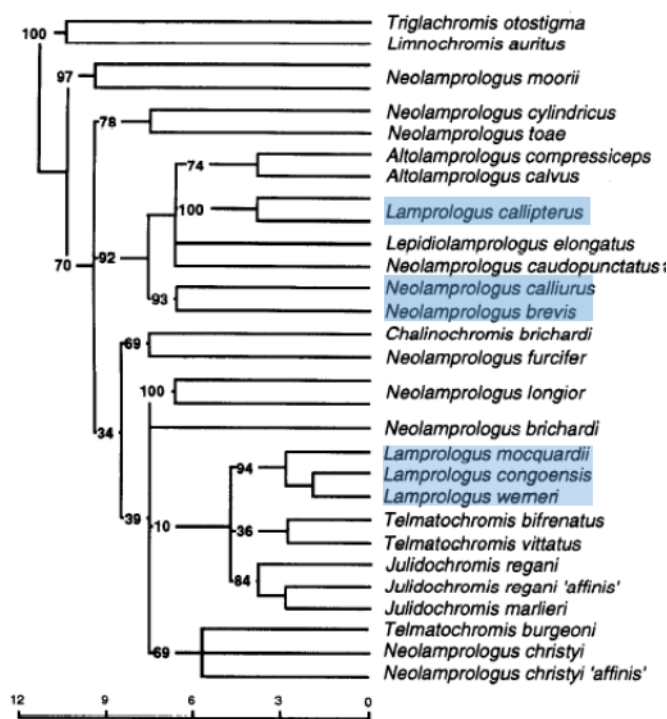


Figure 4: Phylogenetic tree from Sturmbauer et al., 1994. Species considered in this study are highlighted blue.

In Day et al. (2007) the focus was again the phylogenetic relationships between lamprologines, however the study was more comprehensive as it covered approximately 70 species from all seven of the current lamprologine genera. Molecular analysis was performed on mtDNA which has proven reliable for analysing cichlid species relationships (Day et al. 2007). Again phylogenetic trees

were constructed based on the data (Figure 5). The monophyletic nature of Lamprologini was well supported by the data and from analysis three major clades emerged (A, B, and C). The credibility of clade C is questionable, however it does not contain any species of concern for this review. Interestingly the two species of riverine *Lamprologus* considered in the study (*L. teugelsi* and *L. congoensis*) were set solidly in clade B. All other species of *Lamprologus* were sorted into clade A, proposed as *Stiassnia*. *N. brevis* and *N. calliurus* were also set into clade A. There was strong statistical support for the organization of clade A and B and these results were compatible with previous molecular analysis. Day et al. (2007) concluded that there is extensive incongruence between molecular evidence and traditional taxonomy and that further study is needed to resolve this. The plasticity of physical traits in species of cichlids was suggested as a likely reason for this disagreement.

The focus of Sturmbauer et al. (2010) was to determine, through molecular analysis, the evolutionary history of the tribe Lamprologini, rather than to simply resolve the taxonomical interrelation. In this study sequences were taken from mtDNA and from a segment of nuclear DNA. mtDNA samples were sequenced for 77 lamprologine species and nuclear DNA for 42 species. The resulting phylogenetic tree is presented in Figure 6. From this it is apparent that the riverine and lacustrine *Lamprologus* species are not closely related. In fact Sturmbauer et al. (2010) state that the Congo River lamprologines were not recovered as a sister clade and that these species likely diverged from the lacustrine species and are not an ancestral lineage. A review of the *Lamprologus* and *Neolamprologus* genera was also suggested. Despite disagreement between these studies in the placement of all lamprologine species, one point is clear, riverine *Lamprologus* species are distinct from lacustrine. This suggests that the current classification is not accurate and needs to be reworked.

2.4 Morphological Perspective

Historical taxonomy depended heavily on a few physical characteristics, which can be extremely homoplastic, or variable, between individuals (Gashagaza et al. 1995; Sturmbauer et al. 2010). Current molecular methods are more accurate at determining evolutionary relationships as the similarity between the results of all three studies shows. However some morphology also supports the claim that riverine *Lamprologus* species should be separated from lacustrine ones. In 8 lacustrine species a sesamoid (tendon bound) bone is present that is absent in all riverine species (Schelly and Stiassny 2004). A constraint on any conclusions drawn about riverine species is that only four have been sequenced and included in the studies. However the monophyly of Congo River species has been tentatively supported based on physical and molecular characteristics (Schelly and Stiassny 2004).

2.5 Reclassification

It is my suggestion that *Lamprologus* be divided into two genera. Since *L. congoensis* is the type specimen for the genus it is reasonable to give the genus name *Lamprologus* to the riverine species (Simpson 1961). The inclusion of species from the genus *Telmatochromis* into this new *Lamprologus* genus could also be considered, due to the consistent molecular similarity between the two groups. The placement of the other *Lamprologus* species is not uniform throughout the studies and therefore no reasonable conclusions can be drawn on it at this time. The relation between *Neolamprologus brevis*, *N. calliurus* and certain species of *Lamprologus* shell dwellers, specifically *L. speciosus*, was also shown and the creation of a new genus containing these species is plausible. A reclassification of all species in clade A (Figure 5) into the single genus *Stiassnia* has been proposed by Day et al. (2007) however this detracts from the great degree of morphological and behavioural differences shown within the clade (Sturmbauer et al. 2010). Instead the creation of a new genus within clade A could be viable. This new genus would have *N. brevis* as its type specimen and could house the remaining members of lacustrine *Lamprologus* species, until further taxonomical work resolves their placement. There is strong molecular support for this genus containing at least three to four species (*L. speciosus*, *N. brevis*, *N. calliurus*, and potentially *L. ocellatus*). Due to the shell dwelling nature of these species the genus name *Conchalamprologus* (shell-lamprologus) is suggested.

3. Conclusion

The ongoing research into the phylogenetic relationships of lamprologine cichlids has shown that current classification does not match molecular data. There is a large body of evidence to suggest that a comprehensive review of this tribe is required (Day et al. 2007; Schelly and Stiassny 2004; Stiassny 1997; Sturmbauer et al. 1994; 2010). Resolving the *Lamprologus* genus is a key part of this process and will aid in the classification of newly discovered species. The polyphyletic nature of *Lamprologus* has been supported through molecular analysis (Day et al. 2007; Sturmbauer et al. 1994; 2010) and the separation of the riverine species into a separate genus is well substantiated. The creation of a new genus (*Conchalamprologus*) for the current lacustrine *Lamprologus* species and restriction of the genus *Lamprologus* to only riverine species is logical considering current data. The inclusion of *Neolamprologus brevis* and *N. calliurus* into *Conchalamprologus* also follows the phylogenetic data. These steps will further the resolution of Lamprologini. There is a limitation on classifying the riverine lamprologines as only 4 of the 8 species have been sequenced. To fully support the above suggestion the remaining species should also be sequenced and included in any analysis. In addition the Congo River system is poorly sampled and may harbour a variety of undiscovered species. More sampling could help to determine the evolutionary history of riverine Lamprologini and help to determine their origin and place within the tribe.

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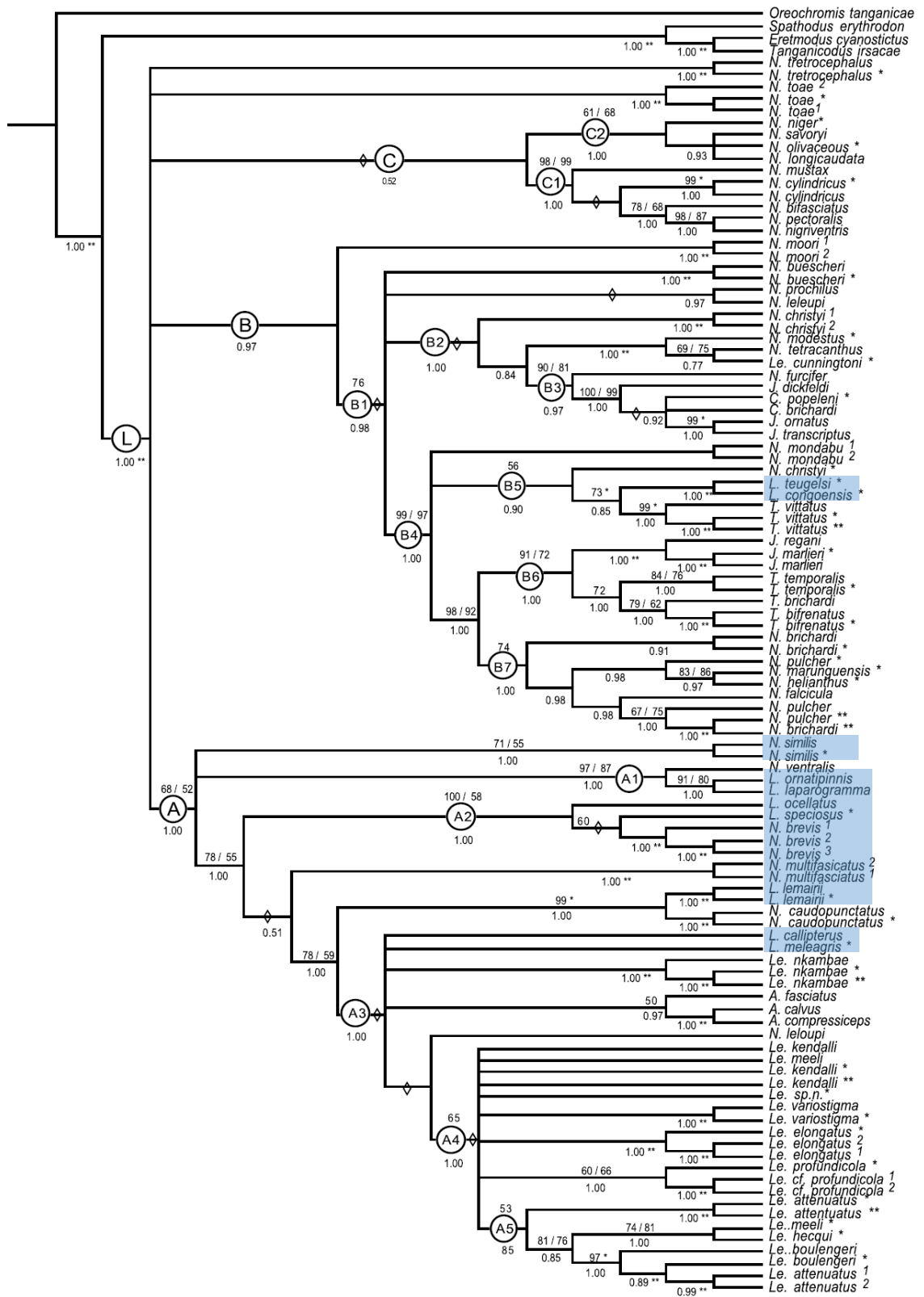


Figure 5: Phylogenetic tree from Day et al. 2007. Species considered are highlighted in blue. L indicates the Lamprologini tribe. A and B are the major clades.

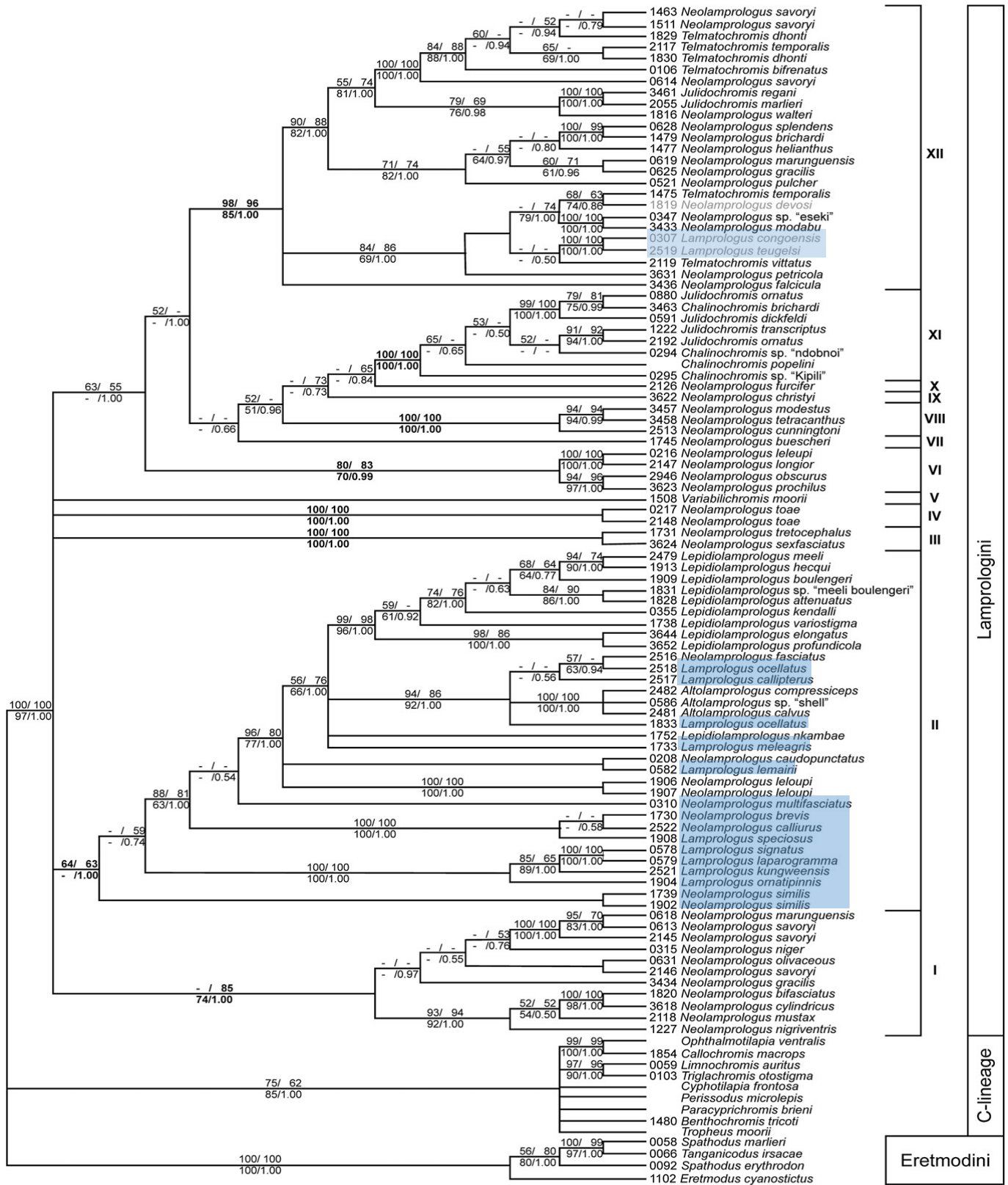


Figure 6: Phylogenetic tree from Sturmbauer et al. 2010. Species considered are highlighted blue.

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