

## Report on DNA studies for taxonomic work

Hartmut Nordsieck (IV.2020)

### Introduction

DNA studies only for taxonomic work are rare. Mostly such studies are made within projects with impressive targets in order to get financial support from the giver institutions. In these projects only one or few species of a group are treated, and related species appear only as outgroups. Thus, DNA-analysed spots of knowledge are generated with large unknown ranges between, not very useful for ordinary taxonomic work.

During the work on a book on European Clausiliidae I was aware that until now several groups of greater importance have not or only incompletely been investigated by DNA studies, though they exhibit severe taxonomic problems. For three of these groups I initiated DNA analyses carried out at my request by molecular working groups (mainly biome-id), financed with own funds saved for this purpose. The first task was the subdivision of the genus *Macrogastra* (with *Julica*), a group which is widely distributed and common in non-meridional Europe (Project 1). The second task was the tribe subdivision of the subfamily Alopiinae, which is the most speciose group of European Clausiliidae and widespread in southern Europe (Project 2). The third task was the genus system of the tribe Delimini which is highly diverse and like other tribes of Alopiinae distributed mainly in southern Europe (Project 3). In all these groups taxonomic work led to problems which could not be solved by morphology alone.

The restricted funds did allow only the examination of a limited number of species and the use of few markers. Therefore, the results of these analyses should be regarded as first approaches; they have to be expanded by future research. None the less they were useful by leading to a better understanding of the phylogeny of the groups.

This is a report on provisional results. The full information on the finished work will be given in the written publication on European Clausiliidae.

### Project 1: *Macrogastra* and *Julica* (Clausiliinae)

DNA studies of the *Macrogastra* group (*Julica*, *Macrogastra*) have been carried out by two working groups, the molecular identification team biome-id (Barco & Knebelberger w. c.) and the Austrian Barcode of Life = ABOL group (Kruckenhauser w. c.). For the trees of the latter mtDNA markers (COI, 16S, 12S), for those of biome-id mtDNA and nDNA markers (COI, H3) have been used. The results of biome-id were presented by a COI-H3 consensus tree (all but one species and two *Clausilia* species included, outgroup *Cochlodina laminata*, Fig. 1). The *Clausilia* species come out in a clade which is the sister group of a well-supported clade including *Julica schmidtii* and all *Macrogastra* species. Within this clade *J. schmidtii* is basal to the *Macrogastra* species which are united in a well-supported clade. Within the *Macrogastra* clade *M. plicatula* is basal to the other species which form a weakly supported polytomy with the following species: *M. ventricosa*, *M. rolphii*, *M. tumida*, *M. badia*, *M. densestriata*, and a well-supported clade including *M. borealis* and a likewise strongly supported subclade *M. attenuata* + *M. asphaltina*.

The polytomy is resolved, if one considers the results of the ABOL group (not figured here). In the COI-16S consensus tree presented by this group *M. rolphii* is basal to a clade including *M. tumida* and the other species (*M. attenuata*, *M. asphaltina*, *M. borealis*, *M. badia*, *M. densestriata*), while *M. ventricosa* is in another clade. In the COI-16S-12S consensus tree presented (in which *M. rolphii* was not included) *M. ventricosa* is basal to the same clade including *M. tumida* and the other named species. In that clade again, *M. tumida* is basal to the other species. It is weakly supported (73%), while the subclade with the other species is well-supported (89%). Within that subclade *M. attenuata*

+ *M. borealis* and *M. badia* + *M. densestriata* come out as sister groups, the first (*M. attenuata* group) strongly supported (100%).

The results are the following:

In the COI-H3 tree of biome-id (Fig. 1) and also in the COI-16S-12S tree of the ABOL group (99% support) *Julica* and *Macrogastra* appear as a monophyletic group. In the COI-H3 tree *Julica* represents the sister group of the *Macrogastra* species; this concurs with its different genital morphology (Nordsieck 1963). Therefore, it is ranked as a genus of its own. In the COI-H3 tree the *Macrogastra* species form a monophyletic group; thus they are assembled in one genus.

Within this genus *M. plicatula* is the sister group of all other species and therefore separated in a subgenus *M. (Pyrostoma)*. This concurs with its male copulatory organs (parepiphallus) different from all other *M.* species (Nordsieck 2006).

Among the remaining species *M. attenuata*, *M. asphaltina* and *M. borealis* belong to a strongly supported monophyletic group. The species *M. densestriata* and *M. badia* are related to that group, because in the COI-16S-12S tree of the ABOL group they form a well-supported clade with this group. That clade represents a new subgenus.

The remaining species of *Macrogastra*, *M. tumida*, *M. rolphii* and *M. ventricosa*, are obviously less closely related to the new subgenus, but *M. tumida* is in the trees of the ABOL group basal to that group within a weakly supported clade. Therefore, it is provisionally included in the new subgenus, while *M. ventricosa* and *M. rolphii* are separated as subgenera of their own (*M. (Macrogastra)* and *M. (Pseudovestia)*, respectively). This is supported by the male copulatory organs of *M. tumida*, which except of their shortened loop resemble those of *M. attenuata*.

## Project 2: Alopiinae

Tribe systems of the Alopiinae based on morphological (mainly genital) characters were proposed by me in several papers since the sixties of the past century (Nordsieck 1969, 1972, 1997).

The DNA analysis of several genera of the Alopiinae by Uit de Weerd & Gittenberger (2013: tree fig. 3) had the following result: *Macedonica*, until now classified with the Cochlodini, was basal to the rest of the subfamily, which came out as monophyletic. This rest was split into two groups, which appeared as sister groups: *Cochlodina* + Delimini and Alopiini (+ *Montenegrina*) + Medorini. Except of *Macedonica*, Alopiinae could thus be subdivided into Cochlodini, Delimini, Alopiini (with *Montenegrina*) and Medorini (as proposed by Bouchet et al. 2017). The weak points of this system, however, were the following: 1. *Macedonica*, represented by only one species, was without tribe affiliation; 2. *Montenegrina* did not fit within the Alopiini, neither by shell nor by genital characters; 3. *Medora*, type genus of the Medorini, and *Strigilodelima* were not included in the analysis. Therefore an expanded DNA analysis of the Alopiinae (three markers: COI, H3, ITS1 of rRNA gene) was carried out by the molecular identification team biome-id (Barco & Knebelberger w. c.). In contrast to that of Uit de Weerd & Gittenberger, several species of the genus *Macedonica* and one each of the genera *Medora* and *Strigilodelima* were included.

The result (consensus tree Fig. 2) was the following: *Macedonica* species form a well-supported clade, basal to the other groups. The rest is distributed among three clades: clade 1 = *Cochlodina*, clade 2 (well-supported) = Delimini, and clade 3 (well-supported), in which several subclades appear as follows: *Herilla*, *Montenegrina*, *Strigilodelima*, *Alopiia*, and Medorini (*Cristataria*, *Agathylla-Muticaria*, *Medora-Albinaria* group). *Herilla* and *Alopiia* are obviously less related as assumed because of their similar morphology; the same is true for *Medora* and *Agathylla*. A comparison with the consensus tree of Uit de Weerd & Gittenberger shows that a well-supported clade with the subclades *Herilla* and *Alopiia-Montenegrina*) = Alopiini sensu Bouchet et al. is not present in the expanded tree of biome-id.

Therefore the following tribe system is proposed: new tribe for *Macedonica*, Cochloclodini, Delimini, and Alopiini (sensu lato), with unnamed subgroups of *Herilla*, *Montenegrina*, *Strigilodelima*, *Alopiia* and former Medorini of about the same rank. To erect tribes for each of these subgroups would certainly be an oversplitting. The system shows that the secondary plicae type of lunellar (lunella more or less reduced, palatal plicae left, clausilium plate notched) has evolved within Alopiinae several times independently.

### **Project 3: Delimini (Alopiinae)**

The genera within the Delimini, tribe of the Alopiinae, were based on morphological characters, mainly of the genital organs, and geographical distribution (Nordsieck 1979, 2002, 2013). With increasing knowledge, however, there arose problems with the delimitation of the genera, because several morphological characters revealed as unsuited for that purpose.

There are no shell characters which can be used for defining genera.

What concerns the genital characters, only few are suited for defining genera. By the different delimitation of the proximal part of penis three groups can be distinguished: *Charpentieria*, *Siciliaria* group (*Siciliaria*, *Papillifera*), and *Delima* group (*Delima*, *Dilatataria*, *Barcania*). It is distinct in *Charpentieria* and absent in the *Delima* group; it is more or less distinct in the *Siciliaria* group. A distinct epiphallar thickening at the transition epiphallus-penis is present in most subgenera of *Siciliaria* (except of *Gibbularia*) and absent in all other genera (Nordsieck 1969); this character could be regarded as an autapomorphy of this genus.

Until now, DNA studies, in which Delimini species are treated (Scheel & Hausdorf 2012, Uit de Weerd & Gittenberger 2013), are incomplete, because only certain species or species groups were included. In the tree of Uit de Weerd & Gittenberger (fig. 3) *Charpentieria* is basal to the other genera; the *Delima* group (*Delima*, *Dilatataria*) and the *Siciliaria* group (*Siciliaria*, *Papillifera*) are sister groups. The expanded tree of Alopiinae of biome-id (see Project 2) had a similar result: *Charpentieria* basal, *Siciliaria* and *Delima* + *Papillifera* as sister groups.

Therefore a DNA analysis of the Delimini (three markers: COI, H3, ITS1) was carried out by the molecular identification team biome-id (Barco & Knebelberger w. c.). In contrast to that of Uit de Weerd & Gittenberger and of Project 2 more species and species of all genus taxa (except of *Barcania*) were included.

The result (consensus tree fig. 3) was the following:

*Charpentieria* and *Papillifera* appear as monophyletic.

The *Siciliaria* group (*Siciliaria*, *Papillifera*) comes out as one clade, but without support. Within that clade *S. (Gibbularia)* and *Papillifera* form a subclade with weak support. *Siciliaria* species from N. W. Sicily appear in different positions: species of *S. (Siciliaria)* in a subclade and a species of another group, *S. (Sicania)*, as a separate lineage. The other subgenera of *Siciliaria* are represented as own lineages by one species each, also the North African *S. (Mauritanica)*.

The *Dilatataria* and *Delima* species have a basal position within the clades; the *Delima* species appear in two different clades.

Therefore, at time the current system of Delimini has not to be altered. *Charpentieria* and *Siciliaria* should be separated as genera, as already proposed in a former paper (Nordsieck 2013). *Delima (Piceata)* has to be transferred to *Siciliaria*, as also proposed in that paper; because of its genital characters (distinct papilla base) it is regarded as a subgenus of its own. The former subgenus *S. (Siciliaria)* should be divided into two subgenera, *S. (Siciliaria)* and *S. (Sicania)*, though this is not supported by morphological differences. *Mauritanica* is confirmed as a subgenus of *Siciliaria*.

Obviously, *S. (Stigmatica)* and *Delima* need further research, because the species classified with come out in different positions.

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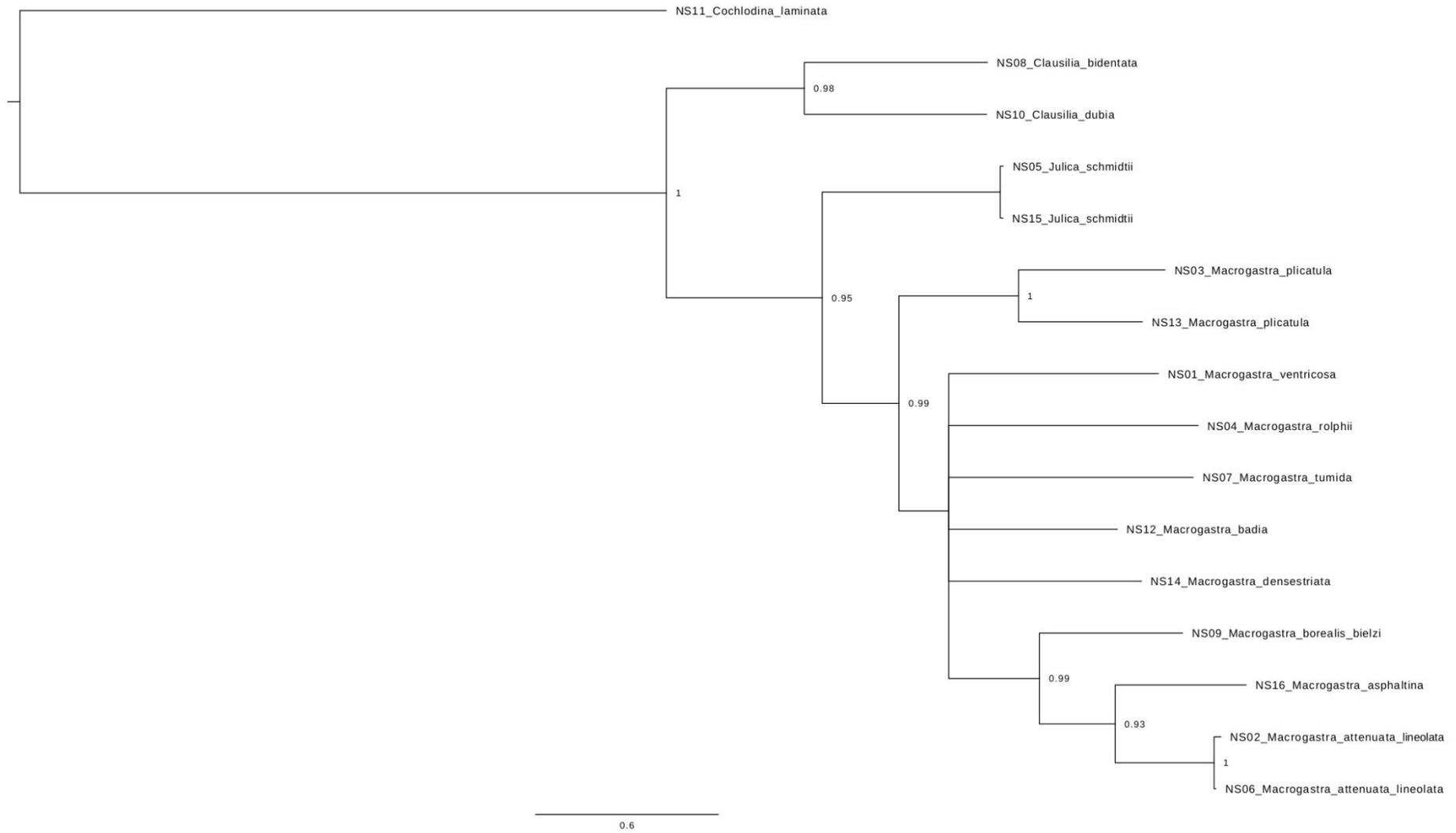
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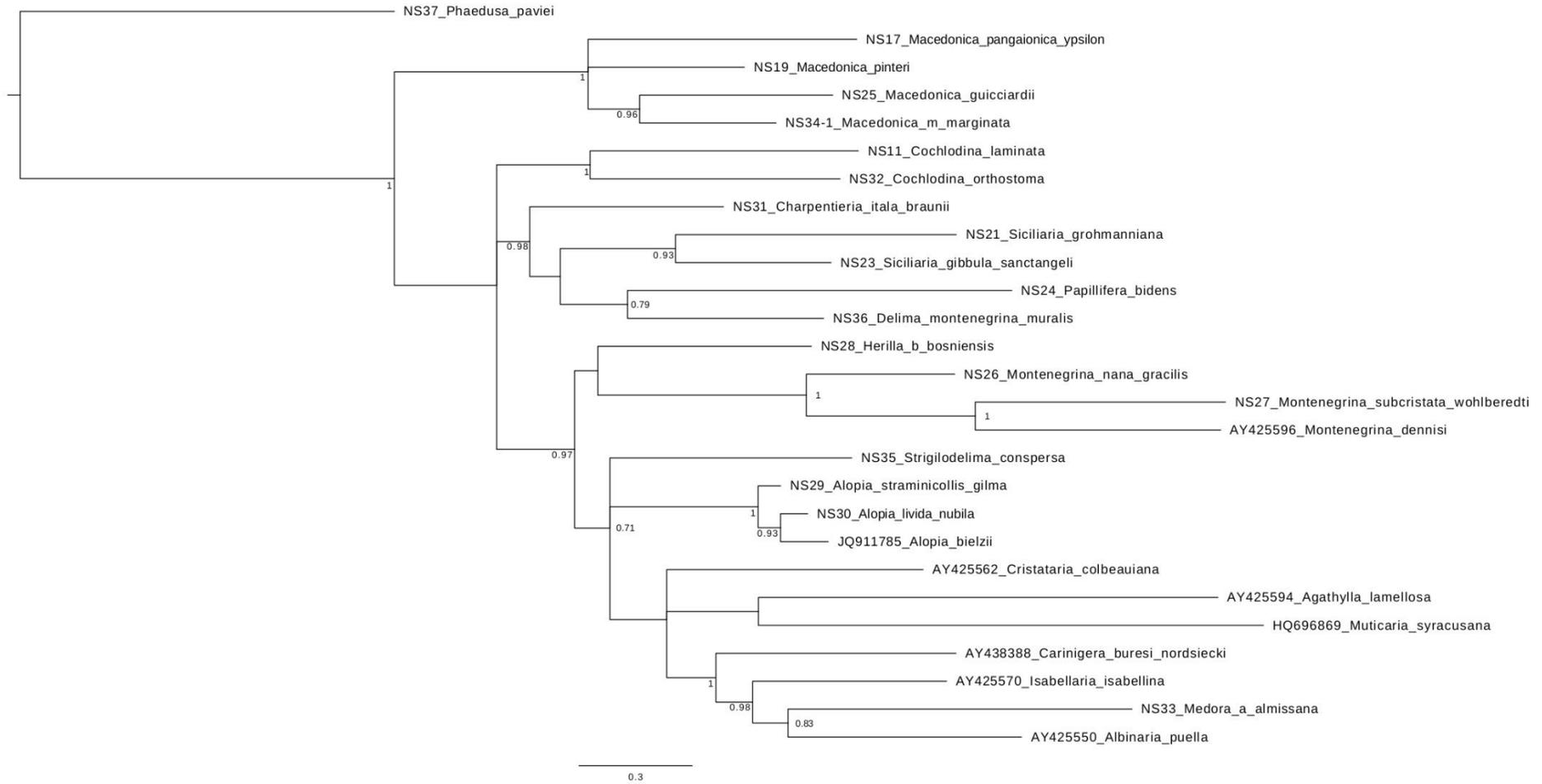
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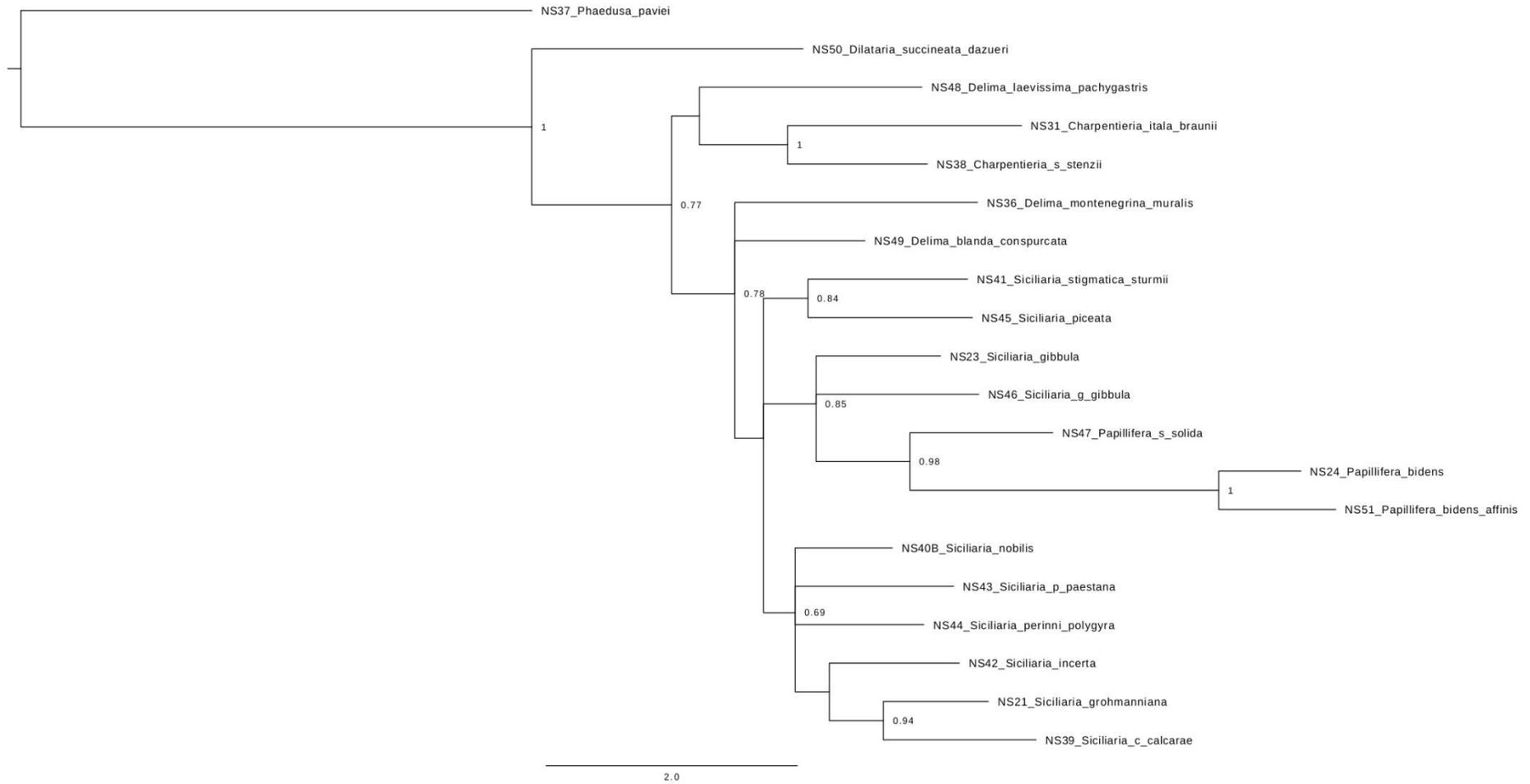
**Project 1: *Macrogastra* and *Julica* (Clausiliinae)**



## Project 2: Aloiinae



### Project 3: Delimini (Alopiinae)



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