

Phylogeny of infraorder Sejina (Acari: Mesostigmata)

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Abstract

Phylogenetic relationships among the families in the infraorder Sejina and the position of Sejina relative to other infraorders of Mesostigmata are re-examined based on molecular and morphological data. Data sets included DNA sequence data for complete 18S, EF-1 α , partial CO1 genes, and 69 morphological characters. The two families of Heterozercnina consistently group within Sejina, and we propose to synonymize Heterozercnina with Sejina (Sejina s.l.). Microgyniina is not the closest relative of Sejina. Rather, Sejina s.l. most often groups with Gamasina. Uropodellidae and Ichthyostomatogasteridae are sister groups and this lineage forms the sister group to Discozerconidae plus Heterozercnidae. Overall, we recognize 5 families within Sejina: Uropodellidae, Ichthyostomatogasteridae, Sejidae, Discozerconidae, and Heterozercnidae.

Key words: Sejina, Sejidae, Heterozercnina, morphology, phylogeny, molecular

Introduction

The infraorder Sejina is an unusual group because it has both a combination of cosmopolitan distribution and a relatively small number of species. About 60 species have been described, many of which have a disjunct distribution. Other widely distributed mesostigmatid infraorders, such as Uropodina and Dermanyssina, are very species rich, while most small infraorders, such as Epicriina and Zerconina, have more restricted distributions. There are no obvious clues in the life history of Sejina. They have been recovered from tree holes, under bark (Hirschmann *et al.*, 1991; Lekveishvili and Klompen, in press), termite nests (Trägårdh, 1906), litter (Balogh, 1963; Athias-Henriot, 1972), bird nests (Hirschmann *et al.*, 1991; Fain and Galloway, 1993), a bat cave (Womersley and Domrow, 1959), and rat nests (Fox, 1947; Athias-Henriot, 1977). Most Sejina are free living although deutonymphs of Sejidae and Uropodellidae are phoretic on beetles, especially on Cerambycidae.

Three families have been described in Sejina: Sejidae (Berlese, 1913) (= Liroaspididae Trägårdh, 1946), Uropodellidae (Camin, 1955), and Ichthyostomatogasteridae (Sellnick, 1953). Sejidae is the most speciose family and includes up to four recognized genera: *Sejus* Koch, 1836 (= *Liroaspis* Banks, 1902; *Dwigubskyia* Oudemans, 1936), *Epicroseius* Berlese, 1905, *Zuluacarus* Trägårdh, 1906, and *Willmannia* Balogh, 1938. Hirschmann (1991), in the most recent revision of Sejidae, synonymized all of these genera in one genus – *Sejus*. Uropodellidae is represented by only one genus - *Uropodella* Berlese, 1888. Two genera have been described in Ichthyostomatogasteridae: *Asternolaelaps* Berlese, 1923 (= *Ichthyostomatogaster* Sellnick, 1953) and *Japanasternolaelaps* Hirschmann and Hiramatsu, 1984.

Family level classification is also unsettled. Uropodellidae was transferred to Ichthyostomatogasteridae by Athias-Henriot (1972). While describing a new genus, *Archaeopodella* Athias-Henriot, 1977, she proposed the additional suppression of the family Ichthyostomatogasteridae and transfer of *Uropodella* and *Asternolaelaps* to Sejidae (Athias-Henriot, 1977). This was based on the assumption that the new genus was intermediate between *Asternolaelaps* and *Sejus*.

At the level of mesostigmatid infraorders, Trägårdh (1946) proposed Microgyniina as the closest relative of Sejina. This hypothesis has been upheld by Camin and Gorirossi (1955) and Johnston (in Norton *et al.*, 1993). Trägårdh (1946) grouped Microgyniina with Sejina based on the presence of several dorsal shields, incompletely fused sternal shields, a slit-like genital aperture, and the absence of an epigynial shield. He placed them in “Agynaspida” along with Megisthanoidea (currently Antennophorina), a group sharing all of these characters except the presence of several dorsal shields. Camin and Gorirossi (1955) noted that female *Liroaspis* (= *Sejus*) have an epigynial shield which is very similar in shape and position to that in gamasines and uropodines. It differs only in the presence of many pairs of setae (only one pair in gamasines and uropodines). Therefore, these mites cannot be regarded as “Agynaspida”. They proposed a new classification with two major lineages, the Trigynaspida with three genital shields in the female, and the Monogynaspida with only one. They placed Liroaspina (= Sejina), Uropodina, and Gamasina in the Monogynaspida. Liroaspina was made up of superfamilies Liroaspoidea and Microgynioidea.

The most recent comprehensive hypothesis of infraordinal relationships (Johnston in Norton *et al.*, 1993) suggests a close relationship of Sejina, Microgyniina, and Uropodina (Fig. 1). Unfortunately, the evidence on which this hypothesis is based has never been stated explicitly, but it is consistent with a major life-history modification. Dispersal in all three infraorders is by deutonymphs attaching themselves to their hosts by anal secretions. This character combination is unknown in any other Parasitiform mite. Kethley (1983) noted that the anal plate structure of *Uropodella* deutonymphs is quite similar to that in the heteromorphic deutonymph of thinozerconoid Uropodina. He hypothesized that the presence of a heteromorphic deutonymph in non-pedicelate uropodines and *Uropodella* is

derivative relative to the condition of having only a single, homeomorph, deutonymphal instar as found in “primitive” Sejina (*Archaeopodella* and *Epicroseius*). He suggested the subsequent loss of the homeomorphic deutonymph in *Uropodella* and higher uropodines. Notably, this idea would imply paraphyly of the Sejina relative to Uropodina. However, species with specialized deutonymphs have since been described in *Epicroseius* (Hirschmann *et al.*, 1991), suggesting that this hypothesis needs adjusting. Also too little is known about *Archaeopodella* to claim no heteromorph.

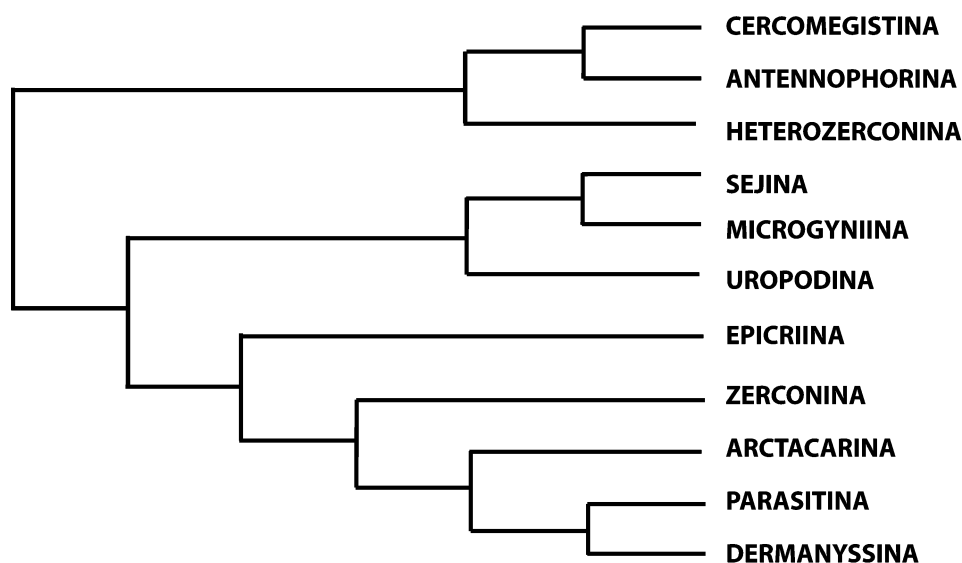


FIGURE 1. Phylogenetic tree by Johnston (in Norton *et al.*, 1993)

In contrast, Klompen (2000) proposed a hypothesis grouping Heterozercnina with Sejina. This arrangement is based on phylogenetic analysis of partial EF-1 α DNA sequence data. However, taxon sampling in this study was relatively poor, including only one representative each of the Sejidae, Ichthyostomatogasteridae, and Heterozercnidae (Heterozercnina). Biologically this grouping is unexpected in terms of both attachment mode and the nature of the associated instar. Adult Heterozercnina are associated with Myriapoda and snakes, holding on with large ventral suckers and pretarsal claws. In contrast, their immature instars do not have suckers and live off the host (Gerdeman *et al.*, 2000). Previous hypotheses of relationships of Heterozercnina are unclear. Johnston (in Norton *et al.*, 1993) grouped Heterozercnina with Trigynaspida, another lineage in which the adult is the dispersal instar, but, as noted above, he did not provide specific evidence. Morphological support for a grouping of Sejina and Heterozercnina is at best weak. Detailed comparison of complete development series of *Sejus carolinensis*

Lekveishvili & Klompen, 2004 and *Narceoheterozercon ohioensis* Gerdeman & Klompen, 2003 (Heterozerconina) failed to generate clear synapomorphies for such a grouping (Lekveishvili and Klompen, in press).

The goal of our study is to use multiple molecular data sets plus morphological data to examine relationships among the families of Sejina and the relative position of the infraorder Sejina within Mesostigmata.

Material

Taxa. The molecular aspect of the study includes 18 representatives of all families of Sejina with four representatives of the family Sejidae. The latter includes members of the two major genera, *Sejus* and *Epicroseius*, and one new species from Great Smoky Mountains National Park (GSMNP), which belongs to a new genus. The morphological part of this study includes additional taxa, selected based on two additional criteria – representation of all recognized genera and species groups (sensu Hirschmann, 1991), and/or availability of all instars. This was achieved for all genera except *Zuluacarus*, which was excluded because of insufficient data for morphological analysis (less than 30% of the characters could be coded).

A wide range of outgroups was selected for the molecular aspects of the study. Representatives of the Microgyniina and Uropodina were included as traditionally recognized close relatives of Sejina (Johnston in Norton *et al.*, 1993; Kethley, 1983). Two heterozerconine species were included to test the result of the EF-1 α study (Klompen, 2000), and representative Trigynaspids were included because of their proposed close relationship with Heterozerconina (Johnston in Norton *et al.*, 1993). Finally, a few Gamasina represent the main diversity of Mesostigmata. *Opilioacarus texanus* (Opilioacarida) was included as primary outgroup, for a total of 18 taxa. Difficulties in establishing homologies over such a broad range of taxa prevented us from using the same outgroup set for the morphological analysis. Only Zerconina, Microgyniina, and Heterozerconina were used as outgroups for these analyses.

Loci. Three different markers were selected including both protein coding and ribosomal genes representing the nuclear and mitochondrial genome: a single copy nuclear protein coding gene - Elongation Factor-1 α (EF-1 α) [1092bp nucleotide, 364 amino acids]; the entire 18S nuclear rDNA [2304 bp aligned] and part of mitochondrial protein coding gene - Cytochrome Oxidase Subunit 1 (CO1) [570 bp nucleotide, 190 amino acids].

Methods

Molecular methods. DNA extractions were performed from a single mite or a few specimens preserved in 95% alcohol using DNeasy[®] Tissue Kit (QIAGEN Inc.) or CTAB

(Black *et al.*, 1997) extractions. Representatives of the same specimen series (secondary vouchers), and the remains of mites after extraction (primary vouchers) are slide mounted and kept at the OSU Acarology Laboratory (OSAL).

Amplification of a target gene region was achieved through the polymerase chain reaction (PCR). Amplification of EF-1 α required nested PCR with initial amplification using primers 40.6F and 41.21RC, followed by another round of PCR using either primer pair 40.71F - M3RC or MF - 53.5RC, amplifying, respectively, the first and second half of the locus. Primers 40.6F, 41.21RC, 40.71F and 53.5RC are from Regier and Shultz (1997), MF and M3RC were designed by authors (MF- 5'-SAR GCH YTN GAY GYN ATG GAR CC-3'; M3RC-5'-GGY TCC ATV RCR TCN ARR GC-3'). The entire 18S gene was amplified in 2-3 partially overlapping parts, using primer combinations NS1-NS2, NS3-NS4, and NS5-NS8 (White *et al.*, 1990) or 1F-5R and 5F-9R (Giribet *et al.*, 1996). Amplification of partial COI used the primer combination P1 (Simon *et al.*, 1994) and R4 (5'-CCW VYT ARD CCT ARR AAR TGT TG- 3').

PCR products were purified using Wizard[®] PCR Preps DNA Purification System (Promega) or QIAquick PCR Purification Kit (QIAGEN Inc). Purified PCR products were sequenced on an ABI PRISM 3700 automated cycle-sequencer (Microbial Plant Genomics Facility, Ohio State University), and assembled using Sequencher 4.1 (Genentech Corp, Ann Arbor, MI, USA). GenBank accession numbers for new sequences as well as for previously published sequences are listed in Table 1 along with voucher specimen numbers.

Alignment of EF-1 α and COI is relatively straightforward, because of the near absence of insertions or deletions and the functional requirements of coding regions. Alignment of 18S rDNA is less intuitive. An initial alignment using ClustalX (Thompson *et al.* 1997) was adjusted manually based on secondary structure (Black *et al.*, 1997; Kjer, 1995). A few highly variable regions, specifically the core of loops 10, e10-1, e23-1, and 49, and part of the region between loops 45 and 46 (224 aligned positions), could not be aligned reliably and were excluded.

Morphology. The morphology based part of the study included 18 taxa and 69 characters. Gnathosomal, idiosomal, and leg characters of all instars were used in the data matrix. The list of characters and data matrix are presented in Appendix 1 and 2, respectively. Characters and their states are discussed in details in a separate study of morphology of Sejidae (Lekveishvili and Klompen, in prep).

Analyses. The total data set included nucleotide sequence data, translated amino acid sequences, and morphology. The amino acid and morphological data matrices were constructed using MacClade v.4.05 (Maddison and Maddison, 2002). The inclusion of both nucleotide and amino-acid sequences might be controversial. For protein coding genes, analyses are generally limited to amino acid sequences, rather than the original nucleotide sequences, when third-codon positions are determined to be saturated. This approach had been used for EF-1 α , for example, by Regier and Shultz (1997, 1998). However, third

codon positions are often phylogenetically informative even when saturation is indicated. In many cases third positions alone find clades with greater stability than do either first or second positions (Wenzel and Siddall, 1999). Amino-acid-sequence characters may “correct” for saturation, but they are subject to convergence that does not affect nucleotide-sequence characters (Simmons, 2000). Given that the information contained in nucleotide and amino-acid sequences is not fully overlapping (Freudenstein *et al.*, 2003) we follow Agosti *et al.* (1996) by incorporating both into a single phylogenetic analyses.

TABLE 1. GenBank accession numbers (new sequences are indicated by *).

Taxa	Abbreviations	Voucher numbers	GenBank accession numbers		
			18S	CO1	EF-1a
<i>Opilioacarus texanus</i>	Opil	OSAL001260	AF124935	-	AF240849
Asternoseiidae	Asts	OSAL004895-97	AY620913	AY623995*	AF256527
<i>Euzercon latus</i>	Euzr	OSAL004892-93	AY620916	-	AY624008*
<i>Microgynium incisum</i>	Micr	OSAL004696-98	AY620919	AY623997*	-
<i>Megisthanus floridanus</i>	Megi	OSAL004894	L76341	AY623996*	AY624009*
<i>Uropoda orbicularis</i>	Urop	OSAL000918-21, 004716-18	AY620926	AY623998*	AY624010*
<i>Prodinychus sp.</i>	Prod	OSAL004728-4732	AY620924	AY623999*	AY624011*
<i>Discozercon sp.</i>	Disc	OSAL004889-91	AY620927	AY624000*	AY624012*
<i>Narceoheterozecon ohioensis</i>	Narc	OSAL003115-16	AY620928	AY624001*	AF256532
<i>Asternolaelaps sp.</i>	Astl	OSAL0414-15	AY620929	AY624002*	AF256530
<i>Epicroseius sp.</i>	Epic	OSAL000416-17, 000588-91, 004722-25	AF287237	AY624003*	AF256531
<i>Sejus carolinensis</i>	S.car	OSAL004642-43, 004637	AY665724*	AY624004*	AF240856
Sejidae GSMNP	GSM	OSAL004587	AY618546*	-	-
<i>Uropodella laciniata</i>	Urpd	OSAL003109-14, 004721	AY620930	AY624005*	AY624013*
<i>Zercon sp.</i>	Zerc	OSAL004898-99	AY665725*	-	AF256533
<i>Proarctacarus oregonensis</i>	Prct	OSAL003312-15	AY620933	AY624006*	AY624014*
<i>Phorytocarpais fime-torum</i>	Phor	OSAL000843-47, 004735-38	AY620935	AY624007*	AY624015*

Separate analyses were conducted for each data set (3 loci, morphology) using parsimony in PAUP*, v.4.0b.10 (Swofford, 2002). These analyses aimed at examining the relative contribution of each data set, not to test for combinability. All parsimony analyses utilized heuristic searches with multiple random additions to avoid local optima (1,000 replicates). All characters were equally weighted. Gaps were treated as missing characters unless otherwise indicated. Jackknife support (Lanyon, 1985) for molecular and combined trees was calculated using options: 37% deletion, emulate "JAC" resampling, 1,000 replications, and the settings "random addition sequences 1" and "hold trees 2", following Freudenstein *et al.* (2004). Support for morphological and combined trees was examined by calculating the Decay Index (Bremer, 1988).

Results

Character interaction. Incongruence Length Difference (ILD) values (Mickevich and Farris, 1981) were calculated to check the level of conflict between data sets. The following interactions were examined: all loci against each other (ILD= 0.8%), each locus and morphology against each other (0.8%), and combined molecular data vs. morphology (0.06%). These values are quite low, suggesting that the various data sets are quite compatible.

Molecular analyses (Fig. 2). *Separate analyses for individual data sets.* The 18S only analysis yielded a single most parsimonious tree (length= 2178; CI= 0.48; RI= 0.42). Based on this tree Heterozercnina groups within Sejina as the sister group to Ichthyostomatogasteridae + Uropodellidae. However, support for this grouping, as well as for sister group relationship between *Asternolaelaps* and *Uropodella*, and for monophyly of the Sejina + Heterozercnina is not very strong. On the other hand, support for monophyly of the Sejidae is strong (100% jackknife support). At the infraordinal level, Microgyniina is not the closest relative of Sejina. Instead, Gamasina appear to be the closest relative of Sejina, although this relationship is only moderately well supported (78%). Microgyniina groups with Uropodina, a relationship that has strong support (100%). Separate 18S analysis where gaps were treated as fifth state produced results (1 tree; length 2277; CI= 0.48; RI= 0.42) that are fully compatible with the previous analysis (tree not provided).

EF-1 α yielded two most parsimonious trees with most branches weakly supported (length= 1502; CI= 0.47; RI= 0.29). Neither Sejina, Sejidae, or Heterozercnina is monophyletic. Monogynaspida are monophyletic (63%) but, Sejina/Heterozercnina is paraphyletic relative to Uropodina and Gamasina. This analysis is thus not consistent with the results of previous EF-1 α analysis (Klompen, 2000) where Uropodina is the sister group of (Heterozercnina + Sejina). It requires 5 additional steps to generate that topology given our data set. EF-1 α sequence data for Microgyniina were not available and this data set therefore cannot be used to address the issue of relationships between Sejina and Microgyniina.

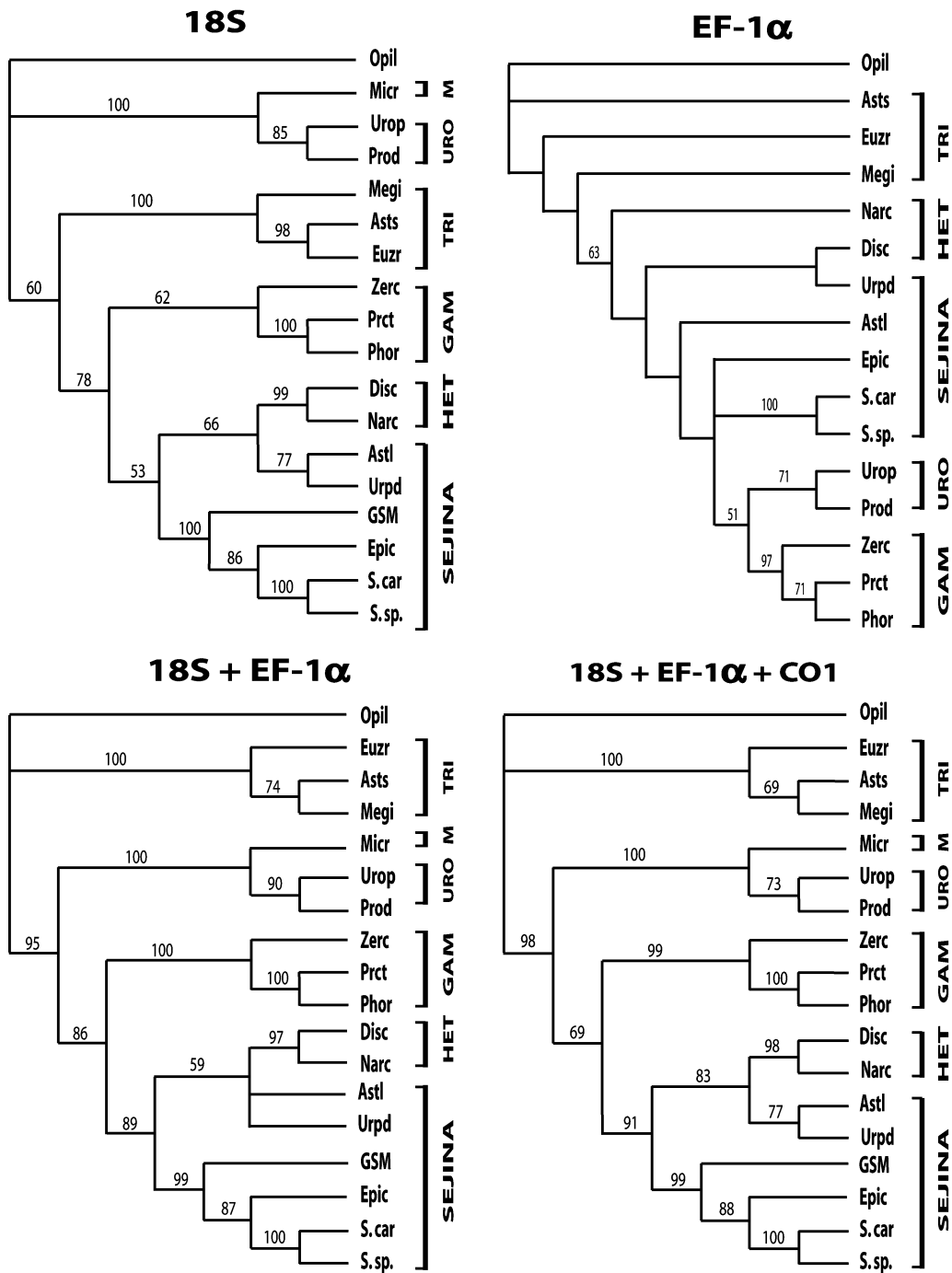


FIGURE 2. Phylogenetic consensus trees based on 18S, EF-1α , 18S + EF-1α , 18S + EF-1α + CO1. Jackknife support is listed above the branches (if >50%). GAM=Gamasina, HET=Heterozeticonina, M=Microgyniina, TRI=Triginaspidae, URO=Uropodina. Other abbreviations as in Table 1.

Most authors (e.g. Cruickshank, 2002) consider the utility of CO1 to be restricted to lower taxonomic levels, that is within species or between closely related species. However, Navajas *et al.* (1996) used this gene to resolve relationships between genera in two families (Tetranychidae and Tenuipalpidae). In our analysis of CO1 resolution was poor. The analysis yielded 4 trees (length= 1048; CI= 0.39; RI= 0.09), and the consensus tree showed only two poorly supported clusters : (Heterozirconina + Sejina) (<50%) and Gamasina (73%) (cladogram not provided). Considering this, we decided to conduct two separate analyses of combined molecular data, one with and one without CO1, to explore whether CO1 contributes anything at all in combined analysis.

Combined analysis of 18S and EF-1 α yielded two trees (length 3739; CI= 0.48; RI= 0.38). Heterozirconina (97%), Sejidae (99%), and the grouping of Heterozirconina within Sejina (89%) are well supported. However, relationships among *Asternolaelaps*, *Uropodella*, and Heterozirconina are not resolved and this entire cluster, while present, is poorly supported (59%). Following the results of the 18S only analysis, Microgyniina is not closely related to Sejina, but is the sister group of Uropodina. Sejina + Heterozirconina appear more closely related to Gamasina.

Combining all molecular data yielded one most parsimonious tree (length 4769; CI= 0.48; RI= 0.36) with most branches well supported. Relative to the 18S + EF-1 α analyses, there is much stronger support for sister group relationships of *Asternolaelaps* and *Uropodella* (77% vs. <50%), and for sister group relationships of that cluster and Heterozirconina (83% vs. 59%). On the other hand, the grouping of Sejina + Heterozirconina, and Gamasina is less well supported (69% vs 86%). It is not clear whether these differences are truly significant, but it appears that the addition of CO1 did improve support and resolution within the Sejina + Heterozirconina branch.

Morphological analysis (Fig. 3). This analysis yielded 4 equally most parsimonious trees (length= 197; CI= 0.49; RI= 0.52). Sejina appears monophyletic, with Heterozirconina as its sister group. However, the latter conclusion may be influenced by the limited number of outgroups included. Within Sejina, *Uropodella* (Uropodellidae) is the sister group of all other taxa, with Ichthyostomatogasteridae (*Asternolaelaps* + *Japanasternolaelaps*) sister-group to Sejidae. Perhaps most surprisingly, *Archaeopodella*, listed as intermediate between Ichthyostomatogasteridae and Sejidae, groups within Sejidae. Excluding it from Sejidae requires 2 additional steps. Notably, overall support levels for these trees are relatively weak.

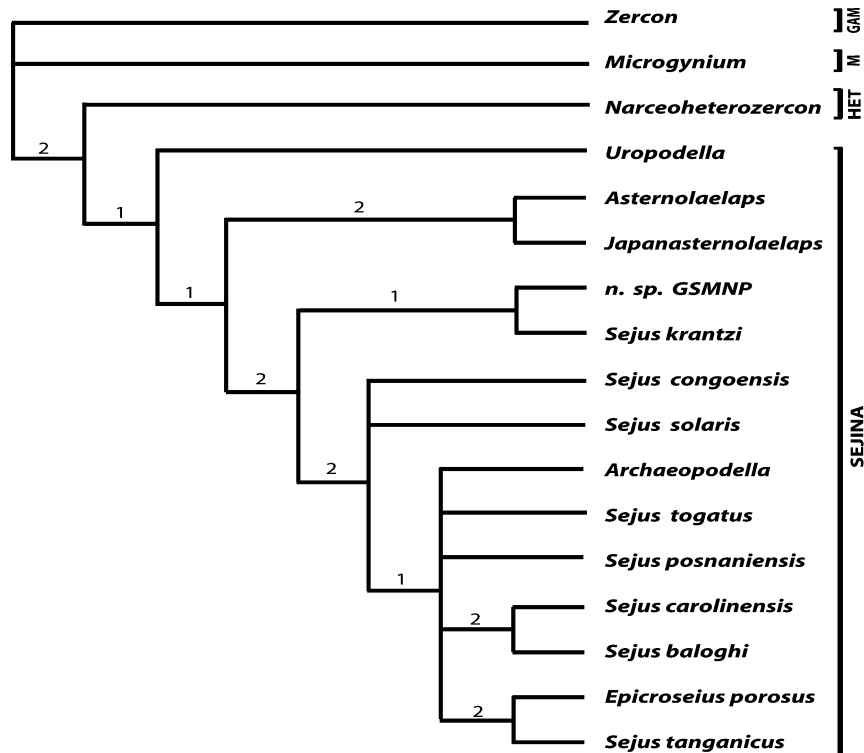


FIGURE 3. Phylogenetic tree based on morphological analysis. Decay index is listed above the branches. Abbreviations as in Fig. 2.

Combined analysis. The initial analysis included all taxa used in either molecular or morphological analyses (total evidence analysis). A total of 13 equally most parsimonious trees (length= 4969; CI= 0.46; RI= 0.34) were generated (Fig. 4A). These results are not influenced by gap treatment. Coding gaps as a fifth state (13 trees; length= 5132; CI= 0.46; RI= 0.35) resulted in a consensus tree with identical topology. These analyses confirm the relationships based on morphological or molecular analyses alone where Heterozerconina are grouped within Sejina and this clade is relatively well supported. Ichthyostomatogasteridae and Uropodellidae are poorly supported sister groups and this cluster forms the closest relatives of Heterozerconina. The infraorder Microgyniina is not the closest relative of Sejina but instead groups close to Uropodina. Finally, the sister group of Sejina + Heterozerconina is Gamasina. Monophyly of Sejidae is recovered, but it is not well supported (1; <50%) and relationships within the group are not resolved.

An obvious problem with the above analysis is the relatively poor taxon overlap for the different data sets, and with high levels of missing data in general. Both molecular and morphological data were available for only a few Sejidae. To test this idea, we conducted another combined analysis including only taxa for which molecular data was available.

This analysis (Fig. 4B) (1 tree; length 4855; CI = 0.48; RI = 0.37) confirms the suspicions on taxon and character sampling. As expected, all groups noted in the total evidence analysis are recovered but with much stronger support.

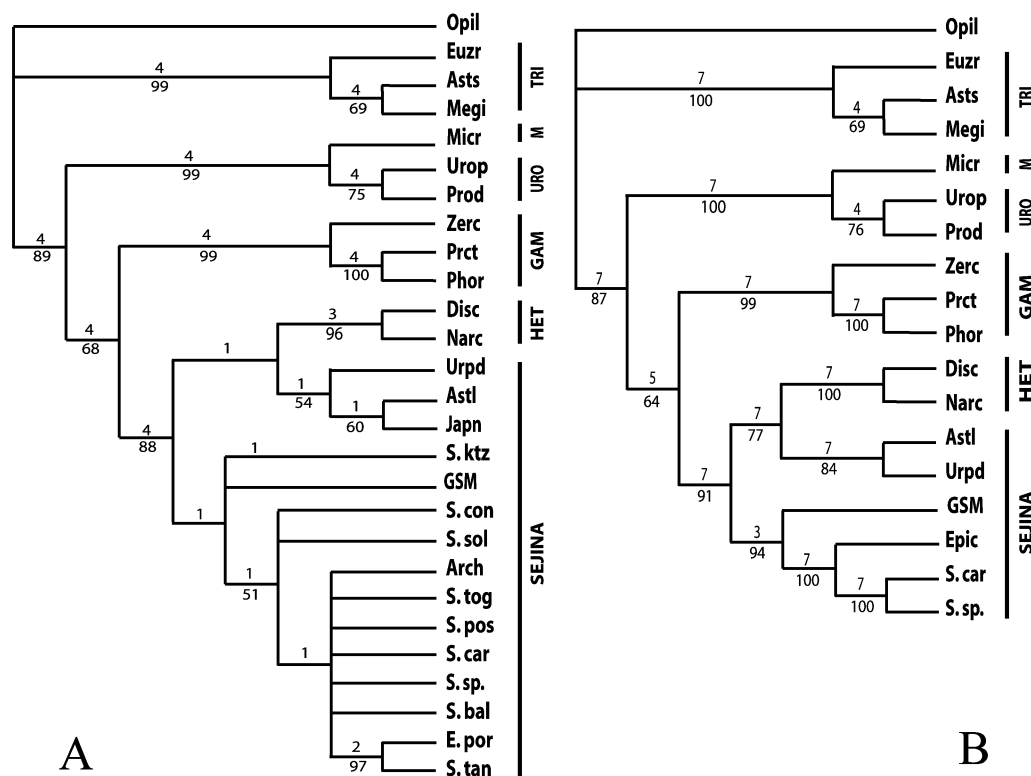


FIGURE 4. Phylogenetic trees based on all molecular and morphological data analyses: A. including representatives of all sejine genera; B. including only taxa with both morphological and molecular data. Bremer support values are listed above and jackknife support below the branches. Abbreviations as in Fig. 2 and Table 1.

Conclusions

Although traditionally Heterozercnina and Sejina were assigned to very distant lineages our study suggests the opposite. Molecular, morphological, and combined analyses support (or at least are compatible with) a close relationship between Heterozercnina and Sejina. Although we could not find any reliable synapomorphies when comparing *Narceoheterzercon* with *Sejus* (Lekveishvili and Klompen, in press), the current morphological data analysis is not inconsistent with the relationship proposed based on molecular data. This clade is strongly supported (91%) by combined molecular analyses and also by the total evidence analyses (7; 91%). Based on these results we propose including Heterozercnina in Sejina (Sejina s.l.).

The traditional hypothesis of a close relationship of Sejina to Microgyniina and Uropodina is not confirmed in our analyses, suggesting that the similarity of attachment organs in phoretic deutonymph is either a homoplasious character, or a shared primitive character, lost in Gamasina and the heterozerconine families. A constraints analysis in PAUP* forcing monophyly of Sejina and Microgyniina (or of Sejina, Heterozerconina, and Microgyniina) required 65 (45) added steps. Similarly, monophyly of Sejina, Microgyniina, and Uropodina (when Heterozerconina is excluded) requires 24 added steps. However, forcing monophyly of these three infraorders + Heterozerconina requires only 6 added steps. This result suggests that a grouping of Uropodina, Microgyniina and Sejina can not be excluded, but only if the heterozerconine families are included.

Based on our results, we recognize five families in the expanded Sejina: Discozerconidae, Heterozerconidae, Uropodellidae, Ichthyostomatogasteridae, and Sejidae. We do not follow Athias-Henriot (1972, 1977) in uniting Ichthyostomatogasteridae, Uropodellidae, and Sejidae in one family. Doing so, and preserving monophyly of all recognized families, would require inclusion of Heterozerconidae and Discozerconidae in Sejidae, an action that seems ill advised. The status of Uropodellidae vs. Ichthyostomatogasteridae is less clear. An argument could be made for continuing synonymy of these two families.

Although monophyly of Sejidae is not recovered by EF-1 α analysis alone, it is supported by 18S, 18S+ EF-1 α , 18S+EF-1 α +CO1, morphological, and total evidence analyses. That being said, the position of *Archaeopodella* in morphological and combined trees is controversial. A broader taxon sampling is necessary to further test the monophyly of Sejidae, and to establish more clear relationships within the family. This issue is being addressed using a morphology based analysis including nearly all recognized sejid species (Lekveishvili and Klompen, in prep).

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ZOOTAXA

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Appendix 1. Morphological characters and character states.

1. Pilus dentilis; [0] present; [1] absent.
2. Gnathotectum; [0] with points; [1] without points.
3. Number of points on gnathotectum; [0]1; [1] 2; [2] 3.
4. Anterior edge of gnathotectum; [0] serrate; [1] not serrate.
5. Shape of gnathotectum; [0] curved; [1] triangular; [2] blunt.
6. Hypostomal seta *hyp1*; [0] setiform; [1] membranous, inflated, with broad base and curved tip; [2] semimembranous, slightly inflated, smooth.
7. Corniculi; [0] horn-shaped; [1] massive, bifid or trifid; [2] flat, lobed, membranous.
8. Lateral dorsal shields in adults; [0] present; [1] absent.
9. Mesonotal shields in protonymph; [0] present; [1] absent.
10. Homeomorphic deutonymph anterior mesonotal shields; [0] partially fused with podonotal; [1] not fused with podonotal.
11. Homeomorphic deutonymph anterior mesonotal shields; [0] fused with each other; [1] not fused.
12. Homeomorphic deutonymph anterior and posterior mesonotal shields; [0] fused with each other; [1] not fused.
13. Homeomorphic deutonymph posterior mesonotal shields; [0] fused with each other; [1] not fused.
14. Homeomorphic deutonymph posterior mesonotal shields; [0] fused with pygidial; [1] not fused.
15. Anterior mesonotal shields in male; [0] fused with podonotal shield; [1] not fused.
16. Anterior mesonotal shields in male; [0] fused with each other; [1] not fused with each other; [2] partially coalesced.
17. Anterior and posterior mesonotal shields in male; [0] fused; [1] not fused; [2] partially coalesced.
18. Posterior mesonotal shields in male; [0] fused with each other; [1] not fused; [2] partially coalesced.
19. Posterior mesonotal shields in male; [0] fused with pygidial; [1] not fused.
20. Posterior mesonotal shields in female; [0] about same size or larger than anteriors; [1] smaller than anteriors.
21. Female anterior mesonotal shields; [0] fused with each other; [1] not fused; [2] partially coalesced.
22. Female posterior mesonotal shields; [0] fused with each other, [1] not fused; [2] partially coalesced.
23. Pygidial shield in larva; [0] present; [1] absent.
24. Pygidial shield in protonymph; [0] present; [1] absent.
25. Pygidial shield in adults; [0] divided; [1] not divided.
26. Posteromarginal shields in adults; [0] present; [1] absent.

27. Posteromarginal shields in female; [0] fused with each other; [1] not fused with each other.
28. Posteromarginal shields in female; [0] fused with pygidial shield; [1] not fused with pygidial shield.
29. Two pairs of projections in larva; [0] present; [1] absent.
30. Two pairs of projections in protonymph; [0] developed [1] not developed.
31. Two pairs of projections in deutonymphs or adults; [0] developed; [1] not developed.
32. Dorsal setae; [0] short and leaf-shaped; [1] not short and leaf-shaped.
33. Marginal setae in adults; [0] five pairs of marginal setae serrate, with expanded tips; at least five times longer than the remaining dorsal setae; [1] all marginal seta about the same length as the remaining dorsal seta and without expanded tips.
34. Lateral extensions of tritosternum; [0] present; [1] absent.
35. Shape of lateral extensions; [0] inverted T-shaped; [1] sickle-shaped; [2] anchor-shaped; [3] barbed.
36. Sides of base of tritosternum; [0] with denticles; [1] without denticles.
37. Surface of base of tritosternum; [0] with denticles; [1] without denticles.
38. 2-3 pairs of dendritic processes lateral of tritosternum; [0] present; [1] absent.
39. Row of denticles posterior to tritosternum; [0] present; [1] absent.
40. Sternal seta *st3* in male; [0] on sternal shield; [1] not on sternal shield.
41. Male sternal region with; [0] five pairs of setae; [1] more than five pairs of setae
42. Male sternal region with; [0] six pairs of setae; [1] seven pairs of setae; [2] nine pairs of setae; [3] more than nine pairs of setae
43. Sternal platelets *st1* in male; [0] fused with each other; [1] not fused with each other.
44. Sternal platelet *st2* in male; [0] fully fused with *st3*; [1] partially fused with *st3*; [2] not fused with *st3*.
45. Seta *st1* in female; [0] on sclerotized platelet; [1] not on sclerotized platelet.
46. Seta *st2* in female; [0] on sclerotized platelet; [1] not on sclerotized platelet.
47. Seta *st3* in female; [0] on sclerotized platelet; [1] not on sclerotized platelet.
48. Seta *st4* in female; [0] on sclerotized platelet; [1] not on sclerotized platelet.
49. Sternal seta *st3* and *st4* in female; [0] on the same platelet; [1] not on the same platelet.
50. Female *st1* platelet; [0] smooth; [1] not smooth.
51. Female *st1* platelets; [0] fused with each other; [1] not fused with each other.
52. Female platelets *st1* and *st2*; [0] fused; [1] not fused.
53. Anterior edge of genital shield; [0] at the middle of coxae II; [1] posterior of coxae II; [2] at the anterior edge of coxae IV.
54. Posterior edge of genital shield; [0] at the posterior edge of coxae IV; [1] behind coxae IV.
55. Female genital shield with; [0] one pair of seta; [1] more than one pair of seta
56. Female genital shield with [0] two pairs of seta; [1] three pairs of seta; [2] five pairs of seta; [3] four pairs of seta

57. Platelets between sternal and ventrianal shields in male; [0] two pairs; [1] more than two pairs; [2] no small platelets.
58. Male genital orifice; [0] presternal, between coxae II; [1] midsternal, between coxae III.
59. The width of female ventrianal shield; [0] about half or less of width of opisthosoma; [1] about 2/3- 3/4 of width of opisthosoma; [2] almost equal to width of opisthosoma.
60. Anus; [0] enlarged; [1] small
61. Ventrianal shield in adults; [0] fully fused with posteromarginal shields; [1] partially fused with posteromarginal shields; [2] not fused with posteromarginal shields.
62. Metapodal shields in deutonymphs or adults; [0] present; [1] absent.
63. Metapodal shields situated; [0] centrally; [1] laterally; [2] shifted dorsolaterally.
64. Metapodal shields in adults; [0] medium size, oval; [1] reduced to two or three mini-platelets; [2] large, triangular; [3] long and narrow.
65. Metapodal shields in male; [0] fused with ventrianal shield; [1] not fused with ventrianal shield.
66. Claws on legs I; [0] absent in immatures and adults; [1] absent in immatures, present in adults; [2] present in immatures and adults.
67. Seta *av4* and *pv4* on tarsus IV; [0] present; [1] absent.
68. Very large and spiniform setae on legs; [0] present; [1] absent.
69. Dorsum of idiosoma; [0] without large glands; [1] with two pairs of large glands; [2] with more than two pairs of large glands.

Appendix 2. Matrix of morphological characters.

Japn — *Japanasternolaelaps japonensis*, Arch — *Archaeopodella scopulifera*, S. ktz — *S. krantzi*, S. con — *S. congoensis*, S. sol - *S. solaris*, S. tog — *S. togatus*, S. pos — *S. posnaniensis*, S. car — *S. carolinensis*, S. bal — *S. baloghi*, E. por — *Epicroseius porosus*, S. tan — *Sejus tanganicus*.

Other abbreviations as in Table 1.

	1	1111111112	222222223	333333334
	1234567890	1234567890	1234567890	1234567890
Zerc	?02000011	000010000-	00?111--11	1111-11110
Micr	10200001?1	101110001-	00??11--??	1111-11110
Narc	01-100201-	----00000-	0011100111	1111-11100
Astl	11-1101011	000000001-	00?011--11	1110311100
Japn	11-11011??	????10001-	11??11--??	1010??0100
Urpd	01-1210011	000010000-	001111--11	1011-00101
Arch	10-0121?01	1111??????	???0?????1	1110311?0?
GSM	01-0000001	000010001-	00?001--?1	1111-11110
S. ktz	00-0100111	000010000-	001011--01	1101-11110
S. con	01-0110110	000010001-	1111101111	1010001101
S. sol	01-011010?	????00001-	0000100101	1101-00100
S. tog	0020110101	1111100000	1110101100	0110211100
S. pos	01-0110101	1111111110	1100101100	1111-11100
S. car	01-0110101	1111111111	1100100000	0110111100
S. bal	01-0010101	1111111111	1110100000	0110111100
E. por	00111101??	????122211	22??0000??	0111-110?0
S. tan	00111101?0	1111122211	22??0000??	0111-110?0

	4444444445	5555555556	666666666
	1234567890	1234567890	123456789
Zerc	0?10000110	00210-210?	-00002112
Micr	0?02000000	00210-2101	-01002110
Narc	0-10000000	110-0--011	21---1100
Astl	4310011011	1-0114-120	201312011
Japn	43000011-1	000114-100	20--10010
Urpd	4302000001	000114-001	-01202001
Arch	??????????	??????????0	?002??011
GSM	100000??0	1010121021	-01012002
S. ktz	0-10010000	1-10102021	01---2002
S. con	0--211001-	--100-1001	202010011
S. sol	32-211000-	--10111001	201012010
S. tog	0-10000001	1110131021	201002010
S. pos	1012000010	1110111001	201012010
S. car	2112000010	1110112001	101112010
S. bal	2112000110	1110131001	101112010
E. por	100011111-	--1010-021	0----0010
S. tan	10-001011-	--1010-021	001010010