

New Family Gulgastruridae of Collembola (Insecta) Based on Morphological, Ecobiological and Molecular Data

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Gulgastrura reticulosa, first described by Yosii (1966) as a monotypic new genus from a Korean limestone cave, was reviewed for its systematic position by cladistic analysis of morphological characteristics, investigation of its intermoult period and reproductive cycle as well as allozyme and 18S rDNA analysis. The great extent of divergence was strongly suggested by its combined lack of sensory organs (third antennal organ, postantennal organ, eyes, pseudocelli) with simultaneous development of an 'apical organ' at the tip of the antennae. The obvious divergence from any existing Collembola families was additionally supported by the extremely prolonged intermoult period as well as by the low strap value it showed with Onychiuridae as obtained by 18S DNA sequence analysis. All these were considered justifying the creation of a new family, Gulgastruridae, but still revealing more allied to Onychiuridae rather than to Hypogastruridae.

Gulgastrura reticulosa was first collected from Gosidong-gul Cave in Kang-won-do Province, Korea, and was described by Yosii as a monotypic new genus (Yosii, 1966). Its systematic position, however, was not clear, having been put in Hypogastruridae, but also considered close to Onychiuridae. Furthermore, the limestone cave, as a type locality, was later transformed to a commercial touristic site and the drastic environmental change rendered the cave animal unavailable for further studies.

It was quite by chance that the first author found it in 1984 from a cave located at Mt. Banryunn-san, Jeonseon-gun in the same province, about 40 Km northwest from the type locality (Lee and Park, 1986). The material was collected only from the entrance of the cave as in the case with the cave of type locality. It enabled the writers to do a series of works on *Gulgastrura reticulosa* of great interest and examine its evolutionary origins. The present paper gives an account of their results and draws the conclusion based on those serial works undertaken so far.

Materials and Methods

Gulgastrura reticulosa was studied under a light microscope as well as scanning electron microscope for character observation. The data were subjected to

cladistic analysis to obtain a cladogram depicting its phylogeny. They were reared to determine the intermoult period and the population dynamics studied to find any clues to their reproductive cycle in operation. Allozymes and 18S rDNA were additionally analyzed for genetic information and pattern which may validate or negate the assumption drawn from morphological data. The multiple approaches used to find out the phylogenetic pathway are described briefly by each of the four different methods employed for the present study.

Cladistic analysis of morphological characters

Under the scanning electron microscope, the absence of the third antennal organ, postantennal organ, pseudocellus, eyes and furca was confirmed, along with the prominent development of an 'apical organ' at the apex of the antenna (Lee and Thibaud, 1987). They all corresponded well with the original description (Yosii, 1966).

To carry out cladistic analysis, specimens of four species, presumably representing each of three families of the superfamily Poduroidea, in addition to *Gulgastrura reticulosa* and three species from three families of the superfamily Entomobryoidea, were collected from several localities of South Korea (Lee et al., 1995b). Twenty eight morphological characteristics were selected for cladistic analysis taking Protura as outgroup to determine the character polarity among Arthropleona groups. The data matrix of transformation series was prepared and analyzed using the PAUP Version 3.0 program.

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The cladistic analysis of morphological characteristics, processed with PAUP, yielded a single, minimum-length tree with 56 steps. In this tree, Arthropleona was subdivided into two branches. In one of them, Hypogastruridae branched first and Neanuridae next, as a sister group of Onychiuridae and *Gulgastrura*. In the other branch, Isotomidae came off first and then Tomoceridae next with Entomobryidae. This result agreed with the traditional classification of Arthropleona divided into two superfamilies, Poduroidea and Entomobryoidea. In the lower category, Tomoceridae has shown to be clustered with Entomobryidae whereas *Gulgastrura reticulosa*, nevertheless, is revealed near Onychiuridae rather than Hypogastruridae.

Ecobiology

The phylogenetic history of *Gulgastrura reticulosa* was also sought against its ecobiological background, that is, moulting behavior and reproductive cycle.

The intermoulting period was revealed to be about 110 d, twice as long as the longest on record (Lee and Thibaud, 1987). This data put forward a strong suggestion that it is fairly divergent from any existing collembolan groups and motivated the first author to check its population change to see whether it gives any clue to the reproductive cycle in operation. This question arised from the assumption that if the problematic species is to be considered a real cave adapted form ('trogllobiont') as suggested by its overall regression of sensory organs, it should not operate any reproductive cycle as normally are the case with true cave forms. The two-year study (Lee and Kim, 1995), however, revealed drastic population drops in June and July and gave a strong indication that *Gulgastrura reticulosa* is to be regarded epigeic in terms of reproductive cycling whereas it is to be a trogllobiont from its extremely modified morphological characteristics, which possibly reflect a dual aspect of mosaic evolution.

Allozyme analysis

The material, the same as used for morphological character analysis, was examined for allozymes as one of the possible indications of genetic affinity among species, thus suggesting a possible phylogeny of the Arthropleona. Twelve allozymes including nineteen loci were investigated for their possible relations (Park et al., 1996) using horizontal gel electrophoresis. This gave rise to allele frequency first and additional data of average number of allele per locus (A), % polymorphism (P), and heterozygosity in observation (Ho). Genetic differences among the seven species under study were also made available by using the NTSYS program.

Finally, a dendrogram was obtained by UPGMA after Sneath and Sokal (1973).

From the allozyme data and values derived from

them, the most prominent features among others were very low variation of *Gulgastrura reticulosa* revealing monomorphism in 86.7% of the loci in contrast to a very high polymorphism in *Crossodonthina koreana* with three fourths of the loci (75%), which is one of the typical epigeic forms of Collembola. The low variation of *Gulgastrura reticulosa* was further made evident by the minimal heterozygosity among species of the Poduroidea superfamily. In general, the number of alleles shared by the species investigated were very small, leading to greater genetic distance among species, and hence, between different families.

In the present allozyme analysis, the way of clustering among the species revealed the same pattern as in the morphological character study; that is, by groupings of families into superfamilies Poduroidea and Entomobryoidea. The same situation occurred with affinities between different families in the superfamilies as well. *Gulgastrura* has grouped with the onychiurid species and not with Hypogastruridae. A similar result was obtained with different coefficients put in UPGMA for producing the dendrogram.

18S rDNA

18S rDNA sequence data were known to be effective in resolving the relationships among crustacean taxa at the intra-order level (Kim and Abele, 1990; Spears et al., 1992). Seven species, presumably representing seven different families of the Collembola order, were used in the same way as in the previous two studies with morphological and allozyme characters. In addition to the first study by the present writer with *Crossodonthina koreana* and *Hypogastrura dolsana* (Hwang et al., 1995), complete sequences were determined for five species by means of PCR cloning and Taq sequencing (Lee et al., 1995a). PCR-Primer sites were located at both ends of the molecule. PCR-reaction was performed for 30 cycles as was done in the senior author's former work (Hwang et al., 1995).

Total length of 18S rDNA of the seven species ranged from 1805 to 1811, indicating that there is little length variation among the species (Lee et al., 1995b). The unrooted tree resulting from the neighbor-joining method using the data matrix prepared showed that the Onychiuridae clustered with *Gulgastrura* and not with Hypogastruridae, which was also the case when the data were processed with maximum parsimony analysis using PAUP, thus supporting familial groupings of the superfamily Poduroidea. The possibility of binding together the former two taxa, however, was observed as low as 50% as the bootstrap value in the dendrogram obtained by neighbor-joining analysis.

Discussion and Conclusion

The phylogeny of *Gulgastrura reticulosa* from a Korean cave was studied with reference to the suborder

Arthropleona in view of its cladistic analysis of external character, moulting behavior, reproductive cycle, allozyme and 18S rDNA analysis. The shifting of systematic position of *Gulgastrura reticulosa* was strongly suggested from the multiple sets of data obtained by four different methods and investigations.

Most of the results are in accord with conventional taxonomy even though a few conflicts were observed with DNA analysis in branching patterns in groupings of families other than with *Gulgastrura*. Since each of the two superfamilies was supported either by Neighbor-joining or Maximum Parsimony Analysis, these data may be another groundset contributing to the consolidating monophyly of the two superfamilies.

The affinity of *Gulgastrura reticulosa* with Onychiuridae was consistently supported by all the different sets of cladistic and experimental analysis. This accords well, even partially, with the original author's perception by stating that "It is to be placed within Hypogastruridae .. But it has apparent similarity to Onychiuridae by the reduction of labral setae..." This view is also supported by the early observation of its mouthparts by Arne Fjellberg (pers.comm.).

Its extensive divergence from any existing Collembola families was obvious as substantiated by the overall regressions of sensory organs, exceptional development of apical organ as well as a very prolonged intermoulting period. It was additionally backed up by the very low bootstrap value as indicator of low binding possibility of *Gulgastrura* with Onychiuridae.

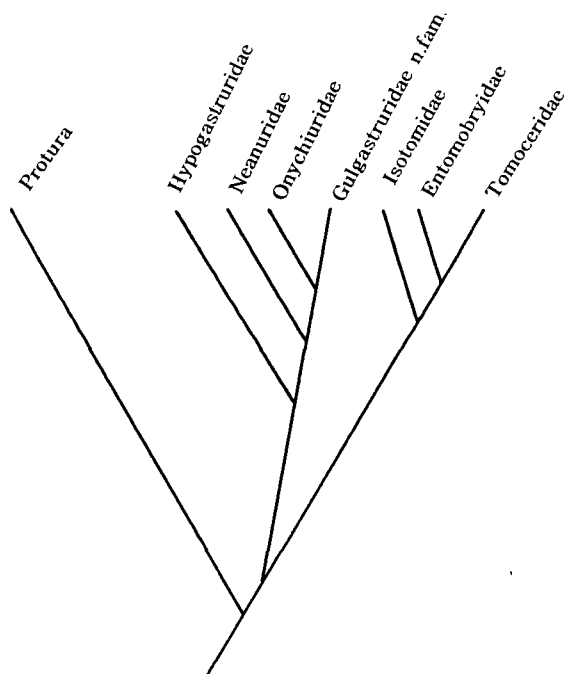


Fig. 1. Phylogeny of *Gulgastruridae* n. fam. with regard to the suborder Arthropleona based on morphological, ecobiological and molecular data analysis.

All these data and interpretation above lead to the conclusion that the cave species in question should be qualified as an independent family and new to science. We give an account of its morphological characters as a new family in the suborder Arthropleona (Fig. 1).

Description

Hypogastruridae n. fam.

Body totally white in color, body length up to 2.5 mm. Body segments well separated, covered with innumerable quantities of setae and integuments reticulated. Third antennal organ (IIIAO), postantennal organ (PAO), eyes and pseudocelli totally absent. At tip of antenna an assemblage of a number of papillae, at least more than 30 in number in a bowl-like cavity, consisting of a few different types of sensillae when seen under the scanning electron microscope. Mandible with well-developed molar plates. Unguis carinate with one inner and a pair of lateral teeth. Unguiculus half of unguis in length with an inner basal lamella. Tenent hair absent. Ventral tube with more than a dozen setae on each side. Furca reduced into two granular tubercles.

Gulgastrura as given as new genus in the original description (Yosii, 1996).

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References

- Hwang U-W, Lee B-H, and Kim W (1995) Sequences of the 18S rDNAs from two Collembola insects: shorter sequences in the V4 and V7 regions. *Gene* 154: 293-294.
- Kim W and Abele LG (1990) Molecular phylogeny of selected decapod crustaceans based on 18S rDNA nucleotide sequences. *J Crustacean Biol* 10: 1-13
- Lee B-H and Thibaud J-M (1987) A critical review of the taxonomy of *Gulgastrura reticulosa* (Collembola: Hypogastruridae), a cave springtail from Korea. *Syst Entomol* 12: 73-79.
- Lee B-H and Park K-H (1986) Three new species of Onychiuridae (Collembola) from a Korean cave. *Korean J Syst Zool* 2: 11-20.
- Lee B-H and Kim J-T (1995) Population dynamics of the springtail, *Gulgastrura reticulosa* (Insecta, Collembola), from a Korean cave. *Spec Bull Jpn Coleopterol* (4): 183-188.
- Lee B-H, Hwang U-W, Kim W, Park K-H, and Kim J-T (1995a) Systematic position of cave Collembola, *Gulgastrura reticulosa* (Insecta) based on morphological characters and 18S rDNA nucleotide sequence analysis. *Mem Biospeol* 22: 83-90.
- Lee B-H, Hwang UW, Kim W, Park K-H, and Kim J-T (1995b) Phylogenetic study of the suborder Arthropleona (Insecta: Collembola) based on morphological characters and 18S rDNA sequence analysis. *Pol Pismo Entomol* 64: 261-277.

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Park K-H, Kim J-T, and Lee B-H (1996) Phylogeny and speciation of Entomobryoidea (Collembola, Insecta) from Korea. *Korean J Syst Zool* 12: 121-136.

Sneath PHA and Sokal RR (1973) Numerical Taxonomy: the Principles and Practice of Numerical Classification. Freeman WH and Co, San Francisco, pp 1-573.

Spears T, Abele LG, and Kim W (1992) The monophyly of brachyuran crabs: a phylogenetic study based on 18S rDNA. *Syst Biol* 41: 446-461.

Yosii R (1966) Results of the speleological survey in South Korea 1966. IV. Cave Collembola of South Korea. *Bull Nat Sci Mus Tokyo* 9: 541-561.

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