

**Two Neanurid Species of Collembola (Insecta) from Korea
with Polytene Chromosomes in Salivary Glands**

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唾腺에 巨大多絲染色體를 갖는 韓國產 흑무늬톡톡이 (昆蟲綱) 2種의 報告

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摘 要

全北의 數個 山地에서 채집된 흑무늬톡톡이 가운데 韓國 未記錄屬으로 *Lobella minuta* n.sp.가 新種으로 확인되었기 이를 記載, 보고한다. 또한 韓國 未記錄屬, 未記錄種으로 이미 그의 唾腺染色體에 관해 보고된 바 있는 (Lee, 1980) *Morulina triverrucosa* Tanaka, 1978의 韓國產 표본에 대해 추가 記載한다. 筆者는 위 新種의 口器가 매우 單純化되어 있음을 관찰하고 Cassagnau의 보고 (1966~1978)에 비취 唾腺의 肥大化와 巨大多絲染色體의 발달 가능성을 추경하게 되었고 이를 檢證하기 위해 細胞 및 組織學的 조사 를 시행한 바 위의 추정이 事實임이 확인되었으므로 그 결과를 발표한다.

Collembola has been recently known to be another insect group with polytene chromosomes in salivary glands, in addition to Diptera, well represented by *Drosophila* for their polytene development and the ensuing great contributions to the understanding of hereditary mechanisms of living organisms. Ever since the first report on the polytene chromosomes in a Collembola species was made by Prabhoo in 1961 one of the French authors, P. Cassagnau, has published a dozen papers concerning their morphology and polymorphism in Neanuridae of this order of insects (Cassagnau, 1966—1978). Collembola, thus, has come to draw special attentions of workers in cytogenetics because it is considered to give new insights into developmental genetics, polymorphism and evolution of insects, particularly from their ametabolous post-embryony as well as consistent maintenance of polytene

chromosomes even in adult stage in contrast to metacloous *Drosophila* where the polyteny is limited to larval period.

For this reason of cytogenetic interests I have come to pay special attentions to Neanuridae group from my routine collections of Collembola because this family has been the only member of Collembola, known to manifest the polyteny in salivary glands. In the course of taxonomical work of identification I was fortunate to find two Neanurid species harboring polytene chromosomes, and that, one of them, new to science and the other, new record to Korea. The holotype and paratypes will be deposited in the Biological Laboratory of Jeonbuk National University in Jeonju, Korea. The followings are the results of my taxonomical, cytological and histological investigations of the two taxa, giving evidences of polytene development and hypertrophy of salivary glands.

Lobella minuta n.sp.

I. Description

Body length up to 1.5 mm. Red in life and white in alcohol. Semi-cylindrical in shape (Fig. 2A) but somewhat oval posture in rest. Tubercles conspicuous, about 70μ in diameter on abdominal tergites, with prominent setae all over the body. Most of setae slightly clavate (Fig. 1B & 2B), longer ones doubling the diameter of tubercles or length of unguis but some in head and tergite margins as well as on abdominal face are of variable length and acuminate. Setae sensulae (s.s.) present mainly in dorso-external tubercles (Fig. 2B). Sixth abdominal segment well bilobed (Fig. 1A). Body surface generally covered with granules except for intersegmental areas and abdominal face, the size of a granule in average 4μ .

Antenna shorter than head diagonal (2:3). Fourth segment ankylosed with the third, thus segment ratio as related about 2:3:6. Apical papilla, three lobed, flanked by 3—4 socket setae. Numerous tapering simple setae present, interposed by 6—7 cylindrical blunt sensillae plus 2 which are less clearly differentiated. Third antennal organ with 2 sense rods, rather clavate and curved in the same direction (Fig. 1D). First and second segments, each with a row of acuminate setae, longer or shorter than segment width.

Ocelli 3+3, of which two situate outside and anterior to ocular tubercle and the remaining one in the latero-posterior portion of the tubercle. Head tubercles well developed and the setae A,B,E,F are tapering macrosetae while C and D are microsetae. Mandible bifurcate but distal branch with three minute terminal teeth, basal one in a short, strong projection and a moderate tooth between the two outer processes (Fig. 1F). Maxilla in stylet form but consisting of two,

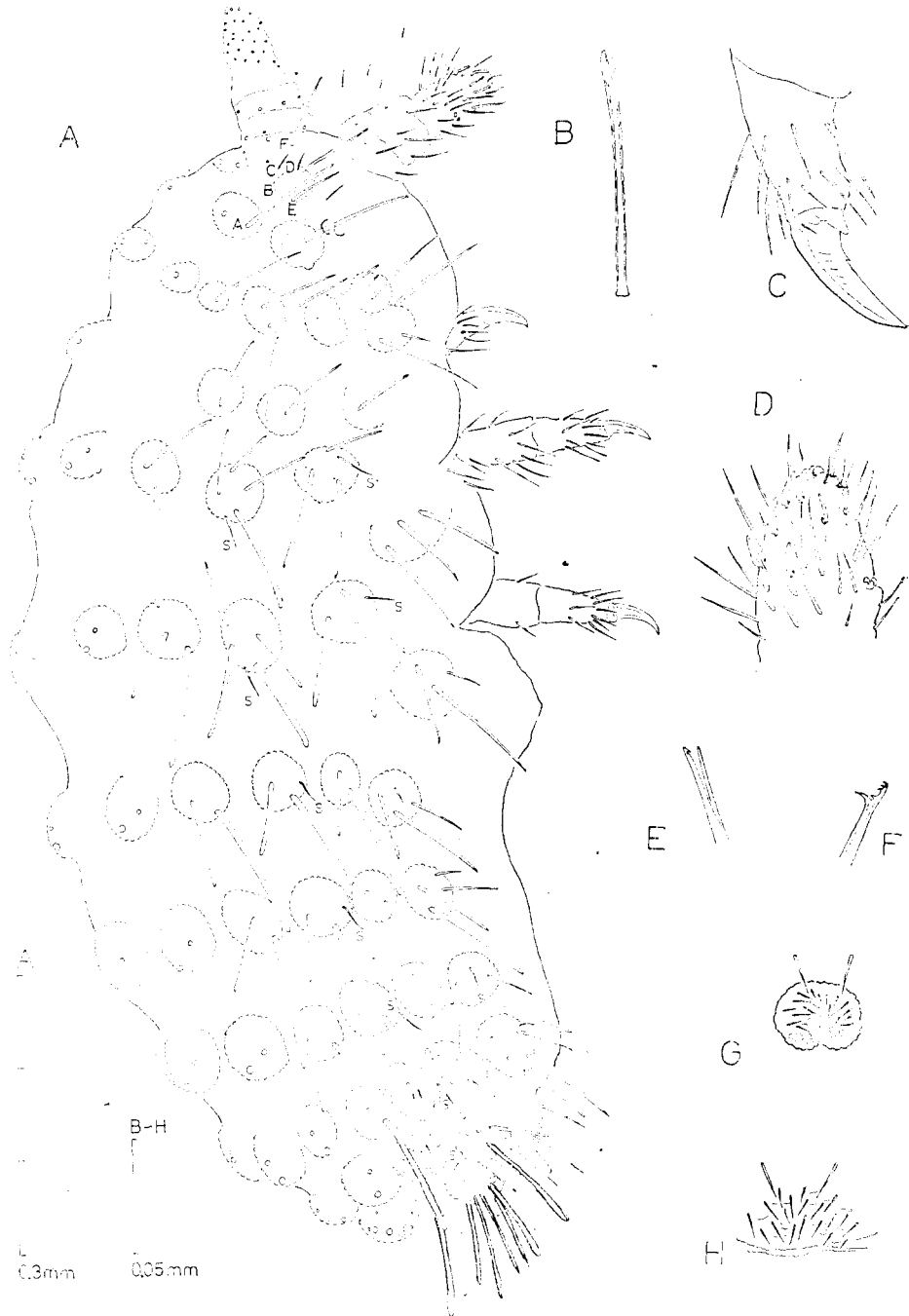


Fig. 1. *Lobella minuta* n. sp.

A: Chaetotaxy of right dorsal side of body, B: Clavate body seta, C: Claw of midleg, D: Antenna segments III-IV., E: Maxilla, F: Mandible, G: Male genital plate, H: Female genital plate.

free, separable lamellae, one of them provided distally with a denticle (Fig. 1E).

Tibiotarsus with two rows of simple setae. Pretarsus with an inner tubercle but no unguiculus. Unguis carinate in external margin, a feeble tooth in the proximal one fourths of the internal margin (Fig. 1C).

Chaetotaxy of the trunk tergites as follows (Fig. 1A).

	DI.	DE.	DL.	L.
Th. I.	1,	2,	1.	
Th. II-III.	2,	4+s,	3+s,	3.
Abd. I-III.	2,	2+s,	2,	3+i.
Abd. IV.	2,	2+s,	3,	4+i.
Abd. V.	2+i,	(4+s.)		
Abd. VI.	7.			

In the Abd. V., dorso-external tubercle coalescent with dorso-lateral one so that a s.s. which would be otherwise in the former tubercle situates at the margin of the fused tubercle, toward the internal median line. Ventral tube with 4+4 setae in lateral flaps. Furcal rest with 3 small setae. Setal arrangement and shape of both ♂ and ♀ genital plate as typical in Neanuridae but provided with two macrosetae above the plates (Fig. 1G & H).

Type data: Holotype ♀. Mt. Namgo-san, Jeonju, Jeonrabug-do Province, from litter sample of a pine stand halfway up the slope (alt. 240 m). Collection no. 79—16. 11, VIII, 1979.

Additional material examined: 21 specimens, same data as holotype.

Remarks: They are well recognized by the prominent setae and tubercles. The nomenclature derives from small size of the body. It was noticeable that they occur always with *Crossondonthina koreana* which belongs to the same subfamily as the present species. However the form most closely related seems to be *Lobella perfusa* Denis, 1948 from Vietnam with which mouth parts fairly resembles but definitely differentiated by chaetotaxy. As far as the tubercles in Abd. V. is concerned it is similar to *Lobella perfusionides* Stach, 1965 from North Vietnam in that dorso-external tubercles in Abd. V. are fused to dorso-lateral one so that s.s. in D.E. tubercle in *Lobella* sensu Yosii is incorporated with the coalescent tubercle. The generic status is nevertheless justified by the partially separable two lamellae of the maxilla (Massoud, 1967). This species, moreover, has been found of particular importance as it carries giant polytene chromosomes in salivary glands as demonstrated in the following investigations.

II. Polytene chromosomes and Salivary Glands

Introduction: As mentioned earlier polytene chromosome in salivary glands have been observed only in some Neanurid Collembola with simple mouth parts. As mandible and maxilla were shown much regressive in the present species I was

intrigued to attempt both cytological and histological tests to know if it is the case with this new taxon as well. In this regard an early effort with a Korean Neanurid species, *Morulina triverrucosa* Tanaka, 1978, was made by me demonstrating the presence of polytene chromosomes and hypertrophied state of the glands as assumed from its simple mouth parts (Lee, 1980). Its occurrence in Korea and a brief account of the external morphology, however, are to be given in this article after the presentation of the present species. The following is the method and the technique employed to investigate the salivary glands and chromosomes, the same as that used for the *Morulina* species of my early study.

Methods: For chromosomal observation specimens were first fixed in Carnoy solution for 24 hours and the integument was removed in the solution with a fine forcep. The head and the prothorax were then cut off from the remaining body trunk and sagittal section was made for these two parts respectively, which gave rise to four pieces in all. Each piece, next, placed on a slide glass and immersed in a drop of 3 N HCl solution was left for one or two minutes and then treated with aceto-lactic orcein solution for staining for 40 minutes. Finally a drop of lactic acid was applied on the tissue sample which was then squashed under a cover glass.

For histological preparation, on the other hand, specimens were first put in the phosphate buffer-formalin mixture (9 : 1, pH. 7.2) for 24 hours and then for the same period of time in running water. They were dehydrated by a series of gradient alcohol solutions from 75% to absolute one and then treated with chloroform. The penetration of paraffin into the tissues was achieved with vacuum infiltrator. The paraffin blocks thus obtained were cut, with rotary microtome, in 5 μ frontal sections which were mounted on slide glasses and treated for coloration with hematoxylin and eosin. After dipping again in alcohol solutions and xylene for partial decoloration they were mounted in Canada balsam on slide glasses which were then ready for microscopic examination.

Results: The squashed preparation first of all showed developed polytene chromosomes with clear bands and interbands, similar to those from *Drosophila* larvae. For technical reasons still unsolved I could not isolate individual set of genome and examine their chromosomal morphology either. It seemed certain, however, that no chromocenters exist. The width of chromosome was measured to be 1 μ in average although swollen regions and puffs were seen, with their widths far more than the average one (Fig. 2D). It was presumed, in view of other cases of similar investigation that constrictions occur while still unclear was if any characteristic structure exist at terminal part of the chromosomes.

As for histological understanding of the salivary gland structures reconstructions of three dimensional form of salivary lobes was attempted using a series of

photographs of the sections on slide preparations. They showed conspicuous hypertrophy and coloration in violet, distinct from other stained portions. No anterior glands at each side of buccal cavity against the integument in *Billobella* species (Cassagnau, 1968) were observed while the posterior glands were extant but differentiated into two lobes: "middle lobe" in the head and "posterior lobe" penetrated into the prothorax, the "anterior lobe" presumably absent or greatly reduced in this species (Fig. 2C). Both middle and posterior lobes were measured about 50–60 μ in diameter but the former consisting of 7–10 cells and the latter, 5–7 cells. The size of a cell in each gland lobe varied from 20–30 μ .

Discussions: As were proved already in a dozen species of Neanurid Collembola by Cassagnau (1966–1978) and in a Korean *Morulina* species of the same family of my study (Lee, 1980) the occurrence of polytene chromosome as well as hypertrophy of the salivary gland seems certainly associated with simple structure of mouth parts although the regressive form of mouth parts would not be the stringent condition of the polytene development as mentioned in my earlier report. In the present species it was noticeable in addition that the structurization of the polytene chromosomes were clearly developed as compared to *Morulina triverrucosa* cited above, by displaying puffs, swollen regions and longitudinal cleavage between homologous chromosomes, for instance, and so on. However the probable absence of chromocenters seems to be consistent with most of previous reports of Collembola cytology, which definitely differentiate them from *Drosophila* group where each chromosome set is tied up by a chromocenter.

Concerning the salivary glands it is assumed that the anterior gland and the anterior lobe of the posterior gland are reduced so that hypertrophy is manifested only in the middle and the posterior lobes of the posterior gland each side. The average size of the cells do not reveal any particular deviance from the data available from previous reports of other taxa, but the number of cells making up the gland lobes differs much and it is to be noted that even in the genus *Lobella* their numbers vary a great deal with different species, for instance, *L. stachi*: 30–40/25–30, *L. perfusa*: 80/80, in the middle and posterior lobes each although they approximate some species of other genera, e.g., *Lathriopyga conjuncta*: 5–8/4–6 and *Neanura muscorum* 10–20/8–15 (Cassagnau, 1968).

In view of the small number of chromosomes ($n=5$ to 9) (Nuñez, 1962), occurrence of puffs and high frequency of inversions of polytene chromosomes (Cassagnau, 1970) cytological analysis of Collembola may be considered a challenging area of study which may lead to new understanding of developmental genetics, evolution and phylogeny of the apterous and ametabolous soil insects as compared to pterygote and metabolous group as represented by *Drosophila* members.

Conclusion: Characterized by the well developed tubercles, long and clavate setae as well as chaetotaxy of the body the present species has been confirmed to be new to science and a genus of new record to Korea. In view of the localities of the related species, that is Vietnam, it seems to have originated from Oriental Region. The simple structure of both mandible and maxilla, moreover, has led me to assume and verify any possible hypertrophy of salivary glands and polytene development and the results suggest a firm validity of the hypothesis of their correlations as were evidenced in earlier reports, including the recent one of mine with a Korean *Morulina* species. It remains to be studied, however, what factors other than simple structure of mouth parts influence the extent of polytene development as were shown between the present species and the *Morulina* species which share almost the same structure and simplicity of mouth parts. It is considered an additional point of interest, moreover, whether structure and number of cells of salivary glands would vary with different taxa, possibly as one of significant taxonomic characters.

Morulina triverrucosa Tanaka, 1978

A brief account of the Korean specimens is as follows.

Description: Body length up to 3 mm. Ground color dark gray. Granules of each tubercle in reticular arrangement (Fig. 4A). The number of tubercles in the trunk tergites is 688/888822. Body setae mostly serrated and even brushy (Figs. 3B & 4C). Sixth abdominal tubercles are, however, not visible above but turned over in the ventral face. Ocelli, 5+5 in number and postantennal organ is in molar shape as generic character (Figs. 3B & 4B). Mouth parts much simple: mandible five-dentate, with three teeth pointed inward in outer distal projection, a strong tooth in inner base and a moderate one between the outer projection and the basal tooth (Fig. 3E). Maxilla in stylet form but consisting of two separable lamellae, each terminated by a denticle or a notch (Fig. 3D). Unguis with a feeble inner tooth (Fig. 3C). Ventral tube with 7–10 small setae in lateral flaps. Furcal rest as two small tubercles with 4+4 setae. Genital plates with 25–30 setae in both sex but that of male characterized by four acuminate setae above the plate (Fig. 3A).

Material examined: 2♀♀, 1♂; Mt. Deogyu-san, Samgong-ri, Seolcheon-myun, Namweon-gun, Province of Jeonrabug-do, at the base, from litter samples of a thicket (alt. 580 m) beside a trout breeding station, Collection no. 77-1-3, 20, VI, 1977. 2♀♀, 1♂, Mt. Jiri-san, Banseon-ri, Sannae-myun, Namweon-gun, Province of Jeonrabug-do, from litter samples of a thicket (alt. 600 m), Collection no. 78-16, 9, IX, 1978. 3♀♀, 2♂♂, Mt. Naejang-san, Naejang-myun, Jeongeub-gun,

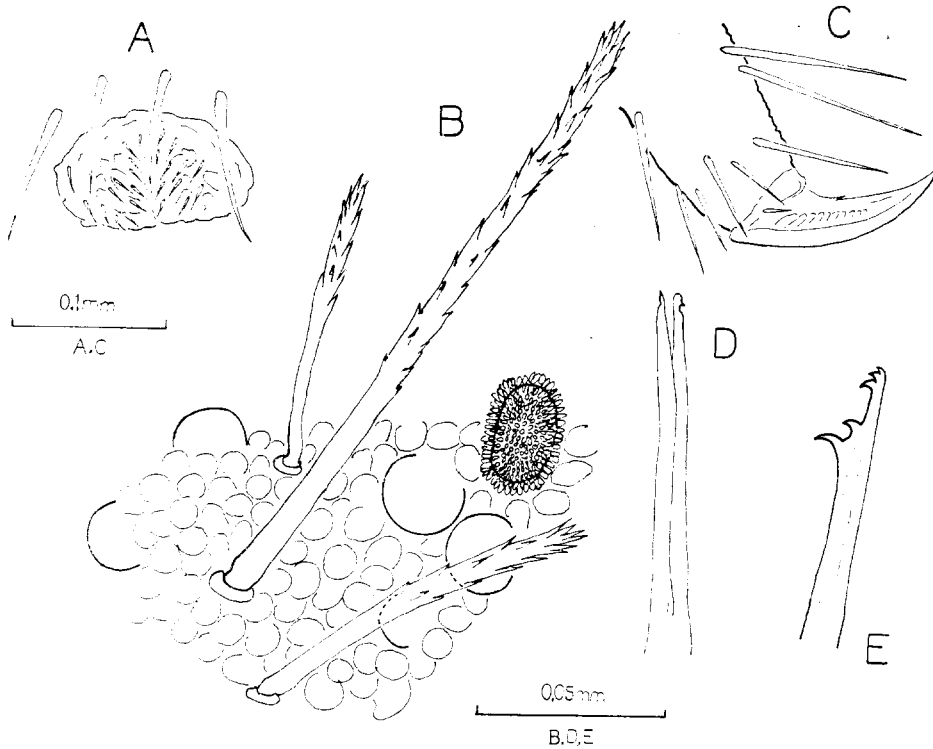


Fig. 3. *Morulina triverrucosa* Tanaka, 1978

A: Male genital plate, B: Ocular tubercle with brushy serrate setae and ocelli, and an adjacent postantennal organ, C: Claw of midleg, D: Maxilla, E: Mandible.

Province of Jeonrabug-do, from litter samples of a thicket at the entrance to the Temple Naejang-sa (alt. 200 m), Collection no. 79-17, 20, VIII, 1979.

Remarks: The characters examined generally agreed well with the original description but body color was different from "purple" in the original account and in chaetotaxy the number of microsetae was more or less than in the Japanese form, in a negligible extent, however, from taxonomical point of view. It remains to be studied, nevertheless, whether the Korean form should be taken as a separate subspecies as more number of specimens would be available in the future, primarily due to the body color difference. It was originally collected and described by Tanaka from limestone areas of Akiyoshi-dai Plateau, North-West Japan. They were from litter samples as in Korea. The present collection marked the first report of the genus *Morulina* from the Korean Peninsula (Lee, 1980).

Polytene chromosomes and Salivary Glands: Both cytological and histological study of salivary glands of the present *Morulina* species was undertaken, as mentioned earlier, precedent to that for *Lobella minuta* of the present paper and

it was demonstrated that even one of the taxa of the Morulini has polytene chromosomes in hypertrophied salivary glands, which is contrary to the former evidences by Cassagnau (1968) where the polytene development had been limited to the tribe Neanurini. However the chromosomal observation of the present species disclosed the structurization far less developed as compared to that of *Lobella minuta* n. sp. as revealed by the subsequent study presented in this paper. Evolutionary implications of the varying extent of polytene development in different taxa were discussed and reviewed relevant to mouth part structures in a few closely related taxa (Lee, 1980).

SUMMARY

Two Neanurid species of Collembola, *Lobella minuta* n. sp., and *Morulina triverrucosa* Tanaka, 1978 were identified from collections of South-Western part of Korea. They were, in addition, confirmed to be genera of new record to the country. From their common structure of simple mouth parts it was postulated that they may have both hypertrophy and polytene development of salivary glands in the light of early evidences by Cassagnau. These assumptions were tested for the above two Korean species using cytological and histological techniques. The results of the investigations have proved to be positive for the postulations, thus supporting hypothetical correlations of simple mouth parts with polytene development, irrespective of taxa, not limited to Neanurini as indicated in early works.

However the structurization of the polytene chromosomes was shown of different extent between the two, i.e., in *Lobella minuta*, clearly more differentiated than in *Morulina triverrucosa* in spite of the strong similarity of mouth parts, which are to be seen as a distinct convergence from two tribes, Neanurini and Morulini. It remains to be studied consequently to know whether there is any factor other than mouth part structure affecting polytene development and also if the parallelism among a few different taxa of mouth parts would be the case with polytene development of salivary glands either.

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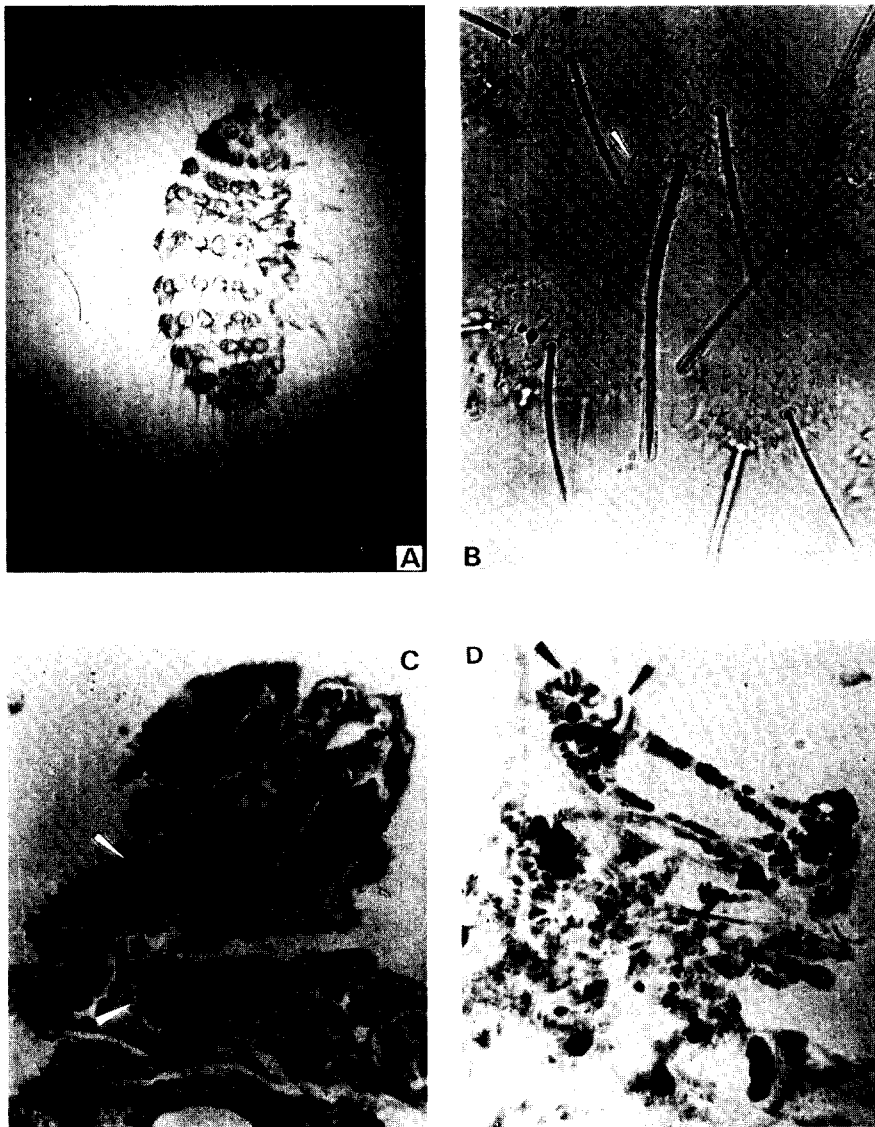


Fig. 2. *Lobella minuta* n. sp.

A: Whole body on slide preparation on which the chaetotaxy of Fig. 1, A is based, B: Abdominal tubercles showing clavate setae and a seta sesulae (arrow) on dorso-external tubercle of Th. II., C: Fronto-sagittal section of the body, showing salivary glands (arrows) in the head and the prothorax, D: Polytene chromosomes showing a swollen banded region and a puff (arrows).

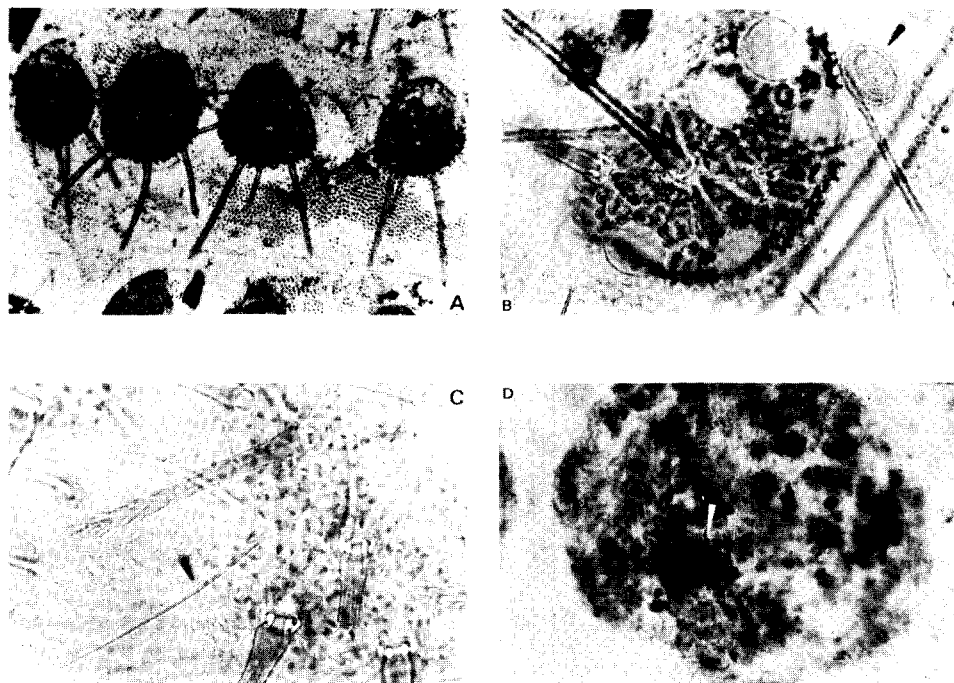


Fig. 4. *Morulina triverrucosa* Tanaka, 1978

A: Tubercles of Th. III., B: Ocular tubercle shown with a postantennal organ (arrow), C: Dorso-external tubercle of Abd. IV showing a seta sensulae (arrow) and a short burshy serrate seta, with basal views of other setae, D: Polytene chromosome (arrow) within a nucleus of salivary gland tissue (For a spread one, see Lee, 1980).