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Corrigendum

Jpn. J. Trop. Med. Hyg., Vol. 25, No.4, 1997, pp. 197-207

1) Nucleotide sequences in Table 2(pp.199) should be corrected like below ;

	erratum		corrected
D4-54S*	AAC-ACA-G**OO**-CTA-ACA-CGC-AT	→	AAC-ACA-G**TT**-CTA-ACA-GTT-T
D4-1978C	ACT-TTT-TCC-TTG-TTT-**FHF**-T**H**	→	ACT-TTT-TCC-TTG-TTT-**ACA**-T**C**
D4-3031C	CTC-TCT-CAA-TAA-CCC-AT	→	CTC-TCT-**ATC**-CAA-TAA-CCC-AT
D4-3674C	A**HH**-AGA-CAT-AGT-GTC-CCC-C	→	A**CC**-AGA-CAT-AGT-GTC-CCC-C

2)Misprint in *Cloning and sequencing of dengue 4 cDNA* (pp.199) should be corrected like below ;

erratum		corrected
5'-CCC-AGT-CAC-GA**H**-GTT-GT-3'	→	5'-CCC-AGT-CAC-GA**C**-GTT-GT-3'

3) Misprint in Table 3 (pp.203) should be corrected like below ;

erratum		corrected
CT93-129		CT93-129
aa (%)		aa (%)
2(1.77)		2(1.77)
3(3.30)		3(3.30)
2(2.67)	→	2(2.67)
25(4.05)		**20**(4.05)
21(5.11)		21(5.11)
48(4.05)		48(4.05)

erratum		corrected
CT93-158		CT93-158
aa (%)		aa (%)
1(0.88)		1(0.88)
4(4.40)		4(4.40)
3(4.00)	→	3(4.00)
19(3.85)		19(3.85)
25(4.87)		**20**(4.87)
42(3.97)		**47**(3.97)

4) Misprint in Table 3 (pp.204) should be corrected like below ;

erratum		corrected
E		E
470 520		470 520
I		I
I	→	I
I		I
D		**M**

5)The numbering format and protein position labels used for the nucleotide and amino acid sequences in Figs. 2 and 3 are not properly aligned.

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STUDIES ON THE CHIRONOMIDAE (DIPTERA, INSECTA) COLLECTED IN MONGOLIA

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Received May 28, 1997/Accepted September 16, 1997

Abstract: Collections of adult chironomids were carried out in Mongolia, Central Asia, located about midway between Europe and Japan, in August 1995 and also in August 1996. The specimens were preserved in 70% ethylalcohol, and individually mounted on slides in gum-chloral medium after digested in 10% hot KOH solution and dissected under stereomicroscope. A total of 345 male specimens were studied for species identification. As the results, a total of 42 species belonging to 23 genera were identified, among which 12 species are considered as belonging to the same one as those already recorded from Europe, and 11 species among them are also in common with those from Japan, and the rest 29 species are described here as new species. Three new genara were created in order to accept 4 new species among them.

Key words: Chironomid midges, Mongolia, Allergens, Asthma, Nuisances

INTRODUCTION

The insects of the family Chironomidae include large numbers of species breeding in various types of lakes, rivers and streams, and have recently attracted special attention as causing a number of problems in the fields of medical and environmental sciences. For example, as was recently reviewed by Sasa (1989), inhalation of dust containing fragments of certain chironomid species acts as the important allergens causing bronchial asthma throughout the world. Such cases were first reported in 1958 from Sudan, where large amounts of a chironomid species, *Cladotanytarsus lewisi*, began to emerge after a manmade lake was constructed on the River Nile, and large numbers of people who moved to the lake-side started to suffer from bronchial asthma. However, it was shown in 1985 by Sasa and coworkers in Japan that many other chironomid species commonly emerging from rice paddies, lakes and rivers are also acting as causes of allergic diseases, and some one-third of the asthmatic patients tested in many rural and urban areas of Japan were shown to be hypersensitive to the chironomid antigens. Furthermore, some chironomids are serious nuisances to many people residing near eutrophicated lakes and rivers all over the world, and necessitates the construction of sewage cleaning plants in many cities, mainly in order to prevent their massive emergence.

On the other hand, large numbers of chironomid species were found to be breeding in various types of lakes, rivers and ditches, and serving as an important factor in the spontaneous removal of pollutants from natural waters. The species of chironomids found in natural waters are quite different according to the chemical and physical characters of the waters, and they are recently utilized as the most excellent biological indicators of the degree of pollution of rivers and lakes.

Extensive studies have been carried out in the European, Oriental, Australian and the Nearctic Regions on the taxonomy, biology and ecology of this group of insects, as was reviewed in the monograph of Chironomidae edited by Wiedelholm (1989). The number of species recorded from the British Islands were about 440 according to Pinder (1978), and that recorded from Japan increased from about 160 in 1978 to more than 800 in 1995, as was reviwed by Sasa and Kikuchi (1995), and some 50 new species are being added every year from this small country. The chironomid fauna of Japan were extensively studies by Tokunaga (1936 a, b, c, 1937, 1940), and since about 1976 by some 50 presently active entomologists. The chironomid fauna of the Oriental Region have also been extensively studied, such as by Makarchenko (1987, 1993, 1994) in Siberia, by Ree (1981) and Ree and Kim (1981, 1988) in Korea, and by Wang *et al.* (1977) and Yan and Ye (1977) in China. However, the chironomid fauna of the Central Asian

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Region have remained almost unknown. Since Mongolia is located between Europe and Japan, the results of the present study carried out for the first time in this country will contribute a great deal to supplement the information on the taxonomy and biology of the family Chironomidae.

This study was conducted as a part of the project No. 07041156, Grants in Aid of International Scientific Research, the Ministry of Education of the Japanese Government, with Prof. Masao Kamiya, University of Hokkaido, as the Project Leader.

Materials and Methods

Collections of adult chironomids were conducted by H. Suzuki during the period from July 30 to August 6, 1995, in and around the capital city of Ulaanbaatur, and from August 6 to August 22, 1996, at around Bogd, about 700 km southwest of the capital. The adult midges were collected during the daytime with insect net while resting in bushes on the shore of lakes and streams, and also at night with sucking tubes while being attracted on electric lamps. The specimens were preserved in 70% ethylalcohol, and were mounted individually on slides in gum-chloral medium following roughly to the method described by Sasa and Kikuchi (1995), but special devices were made this time by Suzuki for the improvements of the methods of mounting the specimens on slides for the taxonomic studies of this group of insects. The methods of descriptions, standard measurements and preparation of figures mostly followed those described by Sasa and Kikuchi (1995), and the technical terms used in the morphological descriptions mostly followed those presented by Pinder (1978) or Saether (1980). The specimens used in the present study, including the holotypes and paratypes, are being deposited in the Institute of Tropical Medicine, Nagasaki University.

Results

As the results of these collections, a total of 345 adult male chironomid specimens were collected and mounted on slides, and they were classified into 42 species belonging to 23 genera, as shown in Table 1. Twelve among them are considered as belonging to the same species as recorded from Europe, and 11 among them are also in common with those recorded from Japan, indicating that they are widely distributing in the holarctic region. The number of species in common with those recorded from Japan but not from Europe is only one. The rest 29 species are described in this paper as new species. The morphological descriptions and figures are presented also to a part of those considered as belonging to the already described species, because they were found to be morhologically somewhat different from the type materials described from Europe or Japan, or in the purpose of supplementing the incomplete original descriptions.

The Species Collected, and Morphological and Taxsonomical Notes

1. *Camptochironomus mongolabeus* sp. nov.

(Figs. 1 a-m)

Five males were collected at Bogd (# 1) on August 13, 1996. Holotype: No. 308:73. Paratypes: 308: 74-77.

Male. In the first 4 specimens with larger body size, BL 10.08-10.90 (10.57 in average of 4) mm, WL 4.34-4.92 (4.58) mm, WW/WL 0.28-0.29, ER 0.12-0.23 (0.18), AR 3.71-4.05 (3.86), AHR 0.52-0.57 (0.55), P/H 0.91-1.11 (1.00), SO 35-44 (40.4), CL 50-70 (59.0), PN all 0, DM 16-36 (24.3), DL 30-50 (30.9). PA 8-11 (9.0), SC 36-56 (45.5), SQ 34-46 (40.0), RR 0.33-0.45 (0.38), VR 1.06-1.11 (1.09), fLR 1.26-1.36 (1.31), mLR 0.50-0.53 (0.52), hLR 0.57-0.60 (0.59). fTR 0.23-0.25 (0.24), fBR 1.7-5.2 (3.7), mBR 1.0-2.3 (1.5), hBR 1.4-3.1 (2.3).

In No. 308:77 with smaller body size, BL 9.96 mm, WL 3.42 mm, WW/WL 0.26, AR 2.90 (smaller), AHR 0.56, ER 0.31, P/H 0.94, SO 32:32, CL 46, PN 0, DM 18, DL 24:26 (smaller), PA 8:8. SC 38, SQ 46:46, RR 0.31, VR 1.05. R/Cu 1.13, fLR 1.57 (larger), mLR 0.53, hLR 0.60, fTR 0.28, fBR 1.1, mBR 1.2, hBR 1.2.

Ground color of scutum yellow, stripes brown, scutellum yellow, postnotum dark brown; femora and tibiae entirely yellow, tarsi I and II largely yellow and apical portion brown, tarsi II to V brown; abdominal tergites almost entirely brownish yellow, hypopygium brown. Head in Fig. 1 a. Eyes bare, antenna with 13 flagellar segments. Frontal tubercles (Fig. 1 b) prominent, almost cylindrical, 43 μm long, 18 μm in diameter, and 45 μm apart from each other. Antepronotum (Fig. 1 c) united in the middle, without lateral seta.Wing bare, venation in Fig. 1 d, typical as a member of the *Chironomus* complex. Tip of front tibia (Fig. 1 e) with a broad and rounded terminal process. Tips of middle and hind tibiae (Fig. 1 f, g) with two comb scales, both with a short spur. Pulvilli well developed, brush-like, claws are simple and with pointed apex.

Abdominal tergites with numerous short setae. Hypopygium in Fig. 1 h, (dorsal, left half) and Fig. 1 i (ventral view, right half). Ninth tergite with a long posterior process flanking anal point, its posterior margin only slightly concave (not deeply concave as in

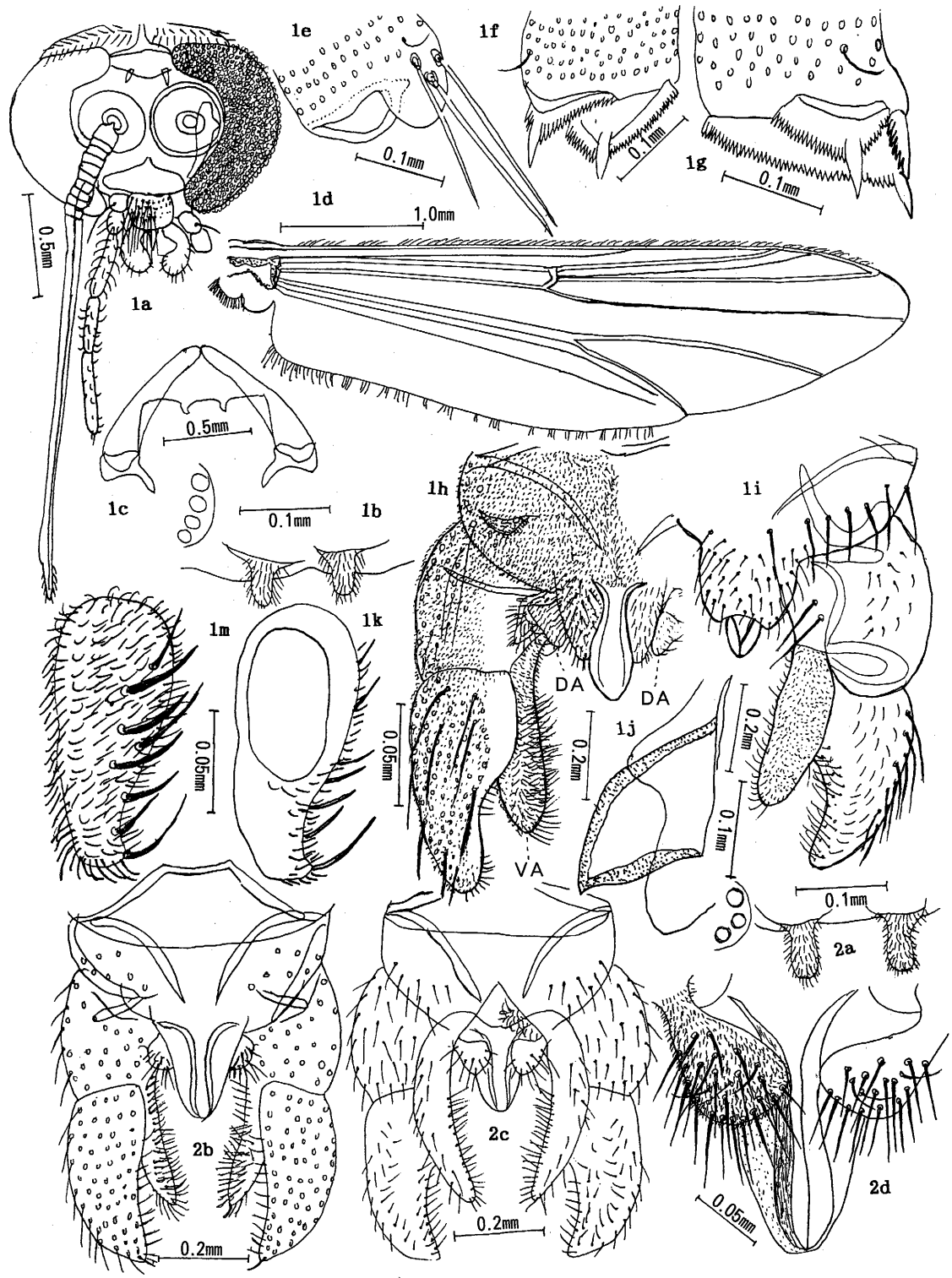

Fig. 1 ***Camptochironomus mongolabeus*** **sp. nov.** a: head, b: frontal tubercles; c: antepronotum; d : wing; e: tip of front tibia; f: tip of middle tibia; g: tip of hind tibia; h: hypopygium, dorsal view, left half; i: hypopygium, ventral view, right half; j: anal point, lateral view; k: dorsal appendage, ventral view; m: dorsal appendage, dorsal view.
Fig. 2 ***Camptochironomus mongolbeceus*** **sp. nov.** a: frontal tubercles; b: hypopygium, dorsal view; c: hypopygium, ventral view; d: anal point and dorsal appendages.

the previously known species). Ninth tergite with a pair of low, broad and rounded lobes laterally (Fig. 1 h). Anal point constricted at base and apically rounded in dorsal view (Fig. 1 h), and somewhat sickle-shaped in lateral view (Fig. 1 j). Dorsal appendage (DA in Fig. 1 h; also in Figs. 1 m, dorsal view, and k, ventral view, both enlarged) elongate oval, smaller and shorter than ventral appendage, with 8 setae on inner margin and dorsal side is entirely clothed with microtrichia. Ventral appendage (VA) very long and finger-like, the tip reaching near tip of gonostylus, with numerous short setae on dorsal side and thickly clothed with microtrichia on both sides. Gonostylus relatively short and very stout, 2.5 times as long as wide, widest near base and apically rounded, with short setae on inner margin.

Remarks. These specimens are considered as belonging to the genus *Camptochironomus* Kieffer, 1918, since anal point is flanked by a process of ninth tergite, dorsal appendage is rather small, elongate oval and clothed with numerous short setae and microtrichia, and ventral appendage is very long, finger-like and almost entirely clothed with numerous short setae and microtrichia (ref. Cranston *et al.*, 1989a). They are closer to *C. pallidivittatus* (Malloch) than to *C. tentans* (Fabricius) among the two European species, in that gonostylus is more robust, but in the present specimens gonostylus is further stouter and shorter (3.0 times as long as wide in *tentans* according to the figure of Pinder, 1978, Fig. 142 B, about 2.5 times in the present specimens). In the above European species, anal point is much more slender, and posterior process flanking anal point is deeply divided into two lobes posterior margin only slightly concave in the present species.

2. *Camptochironomus mongolbeceus* sp. nov.

(Figs. 2 a-d)

A male was collected at Bogd on August 13, 1996. Holotype: No. 308:79.

Male. BL 9.64 mm, WL 4.46 mm, WW/WL 0.28. Ground color of scutum yellow, stripes dark brown, scutellum yellow, postnotum black; femora entirely yellow, tibiae with a conspicuous dark brown apical ring, basal 2/3 of front tarsus I and basal 1/2 of middle and hind tarsi I yellow and their distal portions dark brown, tarsi III, IV and V of all legs entirely dark brown. Frontal tubercles (Fig. 2 a) large, cylindrical. Eyes bare, ER 0.37. Antenna with 13 flagella segments, AR 4.28 (larger than in the former species), AHR 0.69. P/H 1.00. Antepronotum united in the middle, without lateral seta. DM 16, DL 32:32, PA 9:10, SC 21. Wing venation typical as a member of the *Chironomus* complex, SQ 22:24, RR 0.37, VR 1.06, R/Cu 1.16. fLR 1.28 (relatively small), mLR 0.50, hLR 0.59, fTR 0.23, fBR 1.4, mBR 1.7, hBR 1.9. Pulvilli large and brush-like.

Hypopygium in Figs. 2 b(dorsal) and 2 c (ventral view). Anal point flanked by a V-shaped lobe reaching just to tip of anal point, the posterior margin of ninth tergite not concave but convex. Dorsal appendage (Fig. 2 d) much smaller than ventral appendage, highly constricted at base and triangularly expanded, clothed with numerous setae and microtrichia. Ventral appendage extremely long, finger-like, and clothed with numerous short setae and microtrichia, as in the former species. Gonostylus also very wide at base and gradually tapering towards rather pointed apex, also like in the former species, 2.37 times as long as wide, with short setae along inner margin.

Remarks. This specimen also belongs to the genus *Camptochironomus*, since the shape and structure of ventral appendage and gonostylus are quite characteristic and similar to the above species, and ninth tergite seems to have median posterior lobe flanking anal point. It differs from *C. mongolabeus* in that the posterior lobe of ninth tergite is just as long as the anal point and reaches to the tip of it without showing concave posterior margin, and dorsal appendage is quite different in shape, strongly constricted at base and expanded to a broad and triangular lobe bearing numerous long setae and microtrichia. The present species has distinct dark and pale rings on tibiae and tarsi, while femora and tibiae are entirely yellow in the former species.

3. *Chironomus mongolcedeus* sp. nov. (Figs. 3 a-d)

A male was collected at Bogd (# 21), 1,500 m high from sea level, on August 13, 1996. Holotype: No. 308: 51.

Male. BL 6.06 mm, WL 3.46 mm, WW/WL 0.26. Ground color of scutum dark brown, stripes, scutellum and postnotum black, abdominal tergites almost uniformly dark brown, VI, VII and VIII with a narrow pale band along caudal margin, leg segments almost uniformly brownish yellow, excepting tibiae which have short basal and apical dark rings. Frontal tubercles (Fig. 3 a) relatively small, 24 μm long, 12 μm in diameter, and 50 μm apart from each other. Eyes bare, ER 0.35. Antenna with 11 flagellar segments, AR 3.88, AHR 0.71. Palp long, P/H 1.24. SO 26:26, CL 25. Antepronotum black, very narrowly united in the middle, PN 0:0. DM 18, DL 27:28, PA 6:6, SC 28. Wing bare, bluish, anal lobe rectangular, SQ 18:18, RR 0.30, VR 1.05, R/Cu 1.16. fLR 1.41, mLR 0.59, hLR 0.71, fTR 0.21, fBR 2.2, mBR 2.0, hBR 2.8. Pulvilli well developed, brush-like.

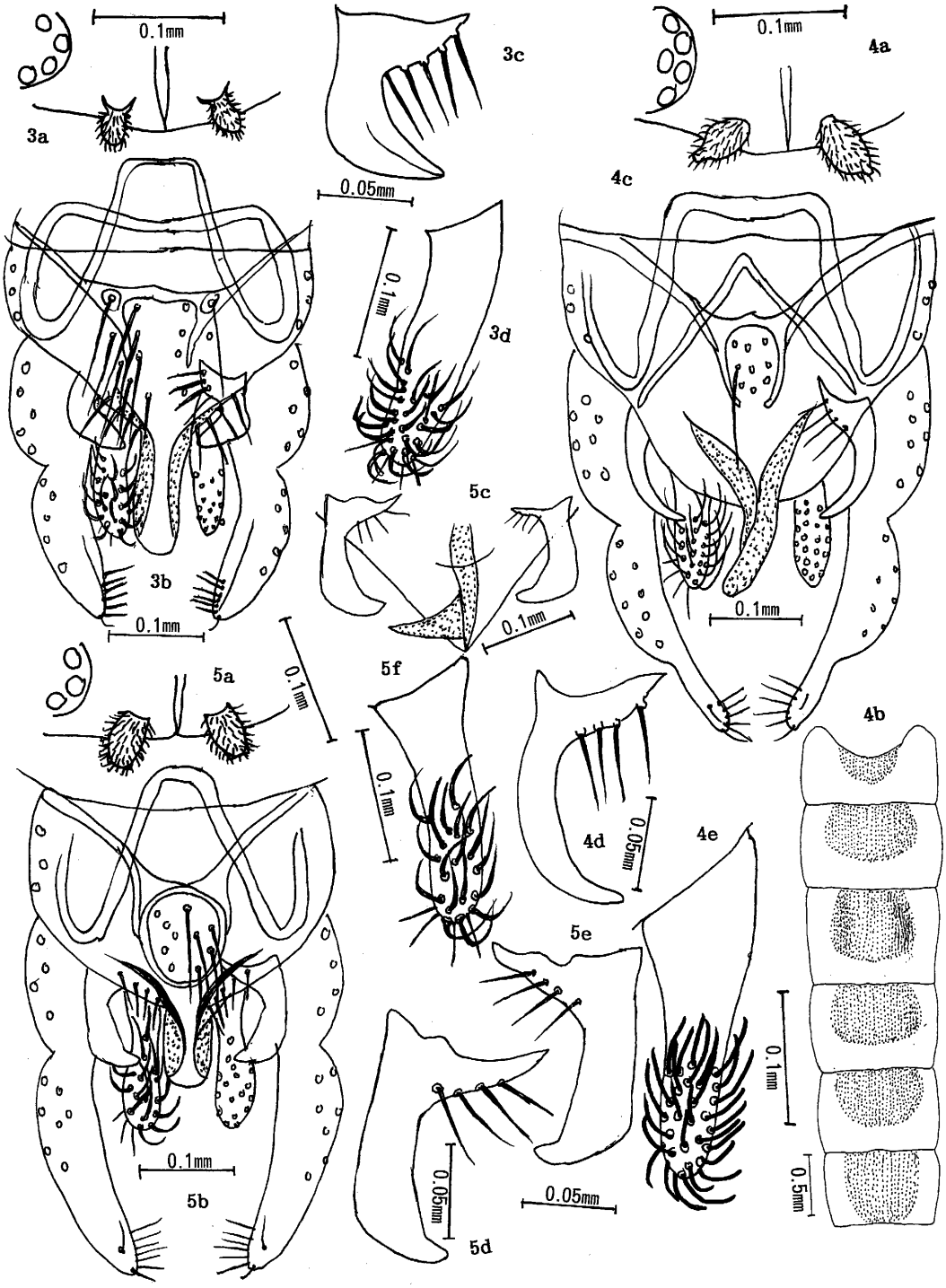

Fig. 3 ***Chironomus mongolcedeus*** **sp. nov.** a: frontal tubercles; b: hypopygium, dorsal view; c: left dorsal appendage; d: right ventral appendage. **Fig. 4** ***Chironomus mongoldeeus*** **sp. nov.** a: frontal tubercles; b: abdominal tergites I-VI, showing dark areas; c: hypopygium; d: left dorsal appedage; left ventral appendage. **Fig. 5** ***Chironomus mongolefeus*** **sp. nov.** a: frontal tubercles; b: hypopygium; c: anal point (lateral view), and dorsal appendages; d: left dorsal appendage; e: right dorsal appendage.

Hypopygium in Fig. 3 b. Anal point stout, parallel-sided and apically rounded. Dorsal appendage (Fig. 3 c) boot-shaped, distal lobe short, stout and rectangularly curved, widest at about middle, and basal portion low, wide and flat, with 5 long inner setae. Ventral appendage (Fig. 3 d) finger-like, parallel-sided and with 24 recurved setae and 4 caudally directed short setae. Gonostylus widest at about middle and not constricted near apex.

Remarks. This species belongs the genus *Chironomus* with S-type (shoe form) of dorsal appendage after Strenzke (1959), and most species of this group have been found breeding in rather polluted sewage waters at least in Japan. However, almost all of them show peculiar pale and dark marks on abdominal tergites, and anal point is narrow and constricted basally, and those found in sulfuric hot spring waters in Japan are all black in body coloration and with narrow anal point (Sasa and Kikuchi, 1995). This species is quite unusual in that abdominal tergites are almost uniformly brown, and anal point is very wide and stout.

4. *Chironomus mongoldeeus* sp. nov. (Figs. 4 a-d)

Twenty one males were collected at Karakorum (# 32) on August 13, 1996. Holotype: No. 308:52. Paratypes: No. 308:53-57, 310:64-78.

Male. Six specimens collected at the same time at Karakorum (# 32) were measured. BL 7.28-11.12 (8.52 in average of 6) mm, WL 3.38-4.81 (3.86) mm, both highly variable, WW/WL 0.27-0.29 (0.28). Ground color of scutum yellow, stripes brown, scutellum yellow, postnotum dark brown, legs almost uniformly yellowish brown. Abdominal tergites II to VIII each with a large brown oval area in the middle surrounded by yellow zones along lateral and posterior margins (Fig. 4 b). Frontal tubercles (Fig. 4 a) widest at about middle and spindle-shaped, 22 μm long, 12 μm in diameter, and 23 μm apart from each other in the holotype. Eyes bare, ER 0.09-0.29. Antenna with 11 flagellar segments, AR 3.48-5.00 (mean, 4.08), AHR 0.53-0.62 (0.58). Palp long, P/H 1.18-1.44 (1.31). SO 32-61 (45.6), CL 13-52 (38.3). Antepronotum united in the middle, without lateral seta. DM 17-28 (22.5: DL 26-51 (34.6), PA 6-8 (7.1), SC 18-46 (33.3), all highly variable.

Wing bare, membrane slightly bluish, R-M darkly pigmented. Costa does not extend beyond tip of R4+5, which is distal to tip of Cul, R/Cu 1.09-1.14 (1.12). R2+3 ending closer to tip of R1 than to tip of R4+5, RR 0.23-0.45 (0.31). R-M slightly proximal to FCu, VR 1.01-1.04 (1.03). Anal lobe slightly produced inwards. fLR 1.32-1.49 (1.44), mLR 0.56-0.62 (0.60), hLR 0.70-0.77 (0.74), fTR 0.21-0.25 (0.23). Beard ratio of tarsomeres I highly variable, fBR 1.9-5.0 (3.1), mBR 2.4-5.3 (3.4), hBR 3.1-6.0 (4.2). Pulvilli well developed.

Hypopygium in Fig. 4 c. Anal point stout, widest at base, and sickle-shaped in lateral view. Dorsal appendage (Fig. 4 d) composed of a low and wide basal portion bearing 4 or 5 inner setae, and a distal horn, which is bare, darkly pigmented, long, slender, smoothly curved and apically pointed. Ventral appendage (Fig. 4 e) long, straight, widest at base and tapering towards rounded apex, bearing some 24 recurved setae arising from the distal half. Gonostylus slender, inner margin slightly concave, and lateral margin abruptly constricted near apex.

Remarks. This and the four following species of this genus collected in Mongol all belong to the group with the E-type (elephant-horn form) of dorsal appendage in the classification of Strenzke (1959). The specimens of this species are highly variable in most measurement data, including body and wing length, AR, LR, BR, and the numbers of setae on head and thorax, but can be identified and differentiated from other species of this genus in the peculiar coloration of abdominal tergites and the peculiar shape of anal point, dorsal and ventral appendages, and gonostylus.

5. *Chironomus mongolefeus* sp. nov. (Figs. 5 a-e)

Three males were collected at Karakorum (# 24) on August 18, 1996. Holotype: No. 308:58. Paratypes: No. 308:59, 60.

Male. BL 7.60, 7.32, 7.64 mm, WL 3.36, 3.40, 3.35 mm, WW/WL 0.28, 0.29, 0.27. Ground color of scutum yellow, stripes largely dark brown and marginal portions black, scutellum yellow, postnotum black, femora largely yellow and distal portion brown; tibiae brown for basal 1/3 and distal portion, the middle portion yellow, tarsi I yellow for basal half and brown for distal half, other tarsal segments brown; abdominal tergites largely brown, II to VIII with yellow band along caudal margin.

Frontal tubercles (Fig. 5 a) prominent, cylindrical. Eyes bare, ER 0.34, 0.30, 0.27. Antenna with 11 flagellar segments, AR 3.61, 3.94, 3.65, AHR 0.67, 0.56, 0.67. Palp long, P/H 1.18, 1.20, 1.13. SO 36:37, 44:46, 34:34, CL 38, 40, 20. Antepronotum narrowly united in the middle, without lateral seta. DM 18, 14, 22, DL 22:25, 34:31, 30:26, PA 7:7, 6:7, 9:9, SC 25, 34, 30.

Wing bare, bluish, R-M darkly pigmented. Squama with 26:26 fringe hairs in the holotype. RR 0.16, 0.17, 0.25, VR 1.01, 0.99, 1.03, R/Cu 1.13, 1.13, 1.12. fLR 1.37, 1.48, 1.39, mLR 0.53, 0.55, 0.53. hLR 0.70, 0.71, 0.71, fTR

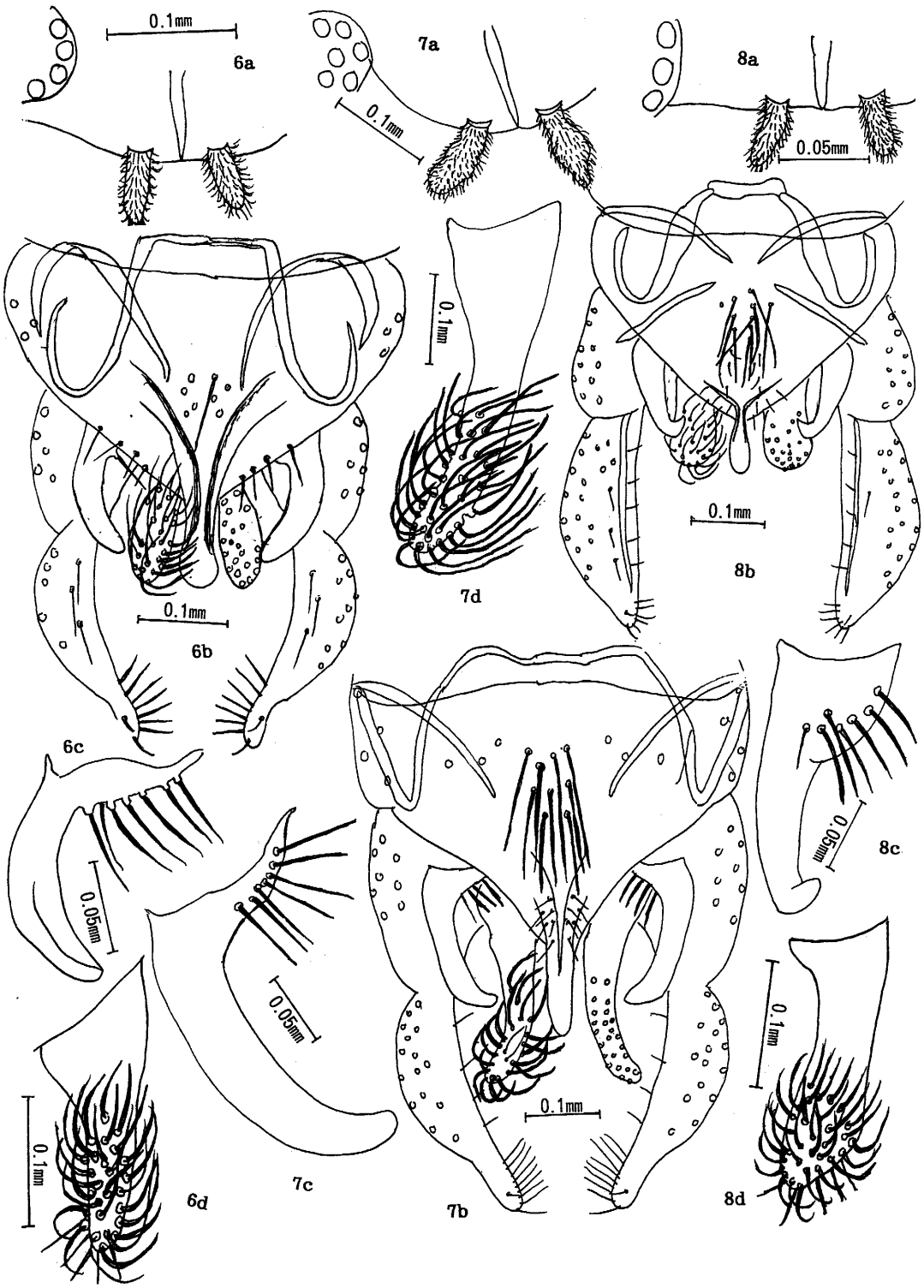

Fig. 6 ***Chironomus mongolfegeus*** **sp. nov.** a: frontal tubercles; b: hypopygium; c: left dorsal appendage; d: left ventral appendage. **Fig. 7** ***Chironomus mongolgeheus*** **sp. nov.** a: frontal tubercles; b: hypopygium; c: left dorsal appendage; d: left ventral appendage. **Fig. 8** ***Chironomus mongolheius*** **sp. nov.** a: frontal tubercles; b: hypopygium; c: left dorsal appendage; d: left ventral appendage.

0.23, 0.24, 0.21, fBR 1.5, 1.4, 2.1, mBR 2.0, 1.8, 5.3, hBR 2.4, 1.8, 4.0, (BR all highly variable).

Hypopygium in Fig. 5 b. Anal point very stout, sickle-shaped in lateral view (Fig. 5 c). Base of dorsal appendage low and broad, with 4 or 5 basal setae, distal horn stout, parallel-sided, inner margin nearly straight but apically hooked inwards (Fig. 5 d, e). Ventral appendage (Fig. 5 f) widest at base, with 18 recurved and 4 caudally directed short setae. Gonostylus slender, widest at about basal 1/3, lateral margin convex and not apically narrowed.

Remarks. This species is somewhat related in the shape of stout anal point to *C. anthracinus* Zetterstedt among the European species of this genus, but differs essentially in the shape of dorsal appendage, which is long, slender and strongly curved in the latter (cf. Pinder, 1978). Three among the Japanese species of this genus have the stout anal point, *C. nipponensis* Tokunaga, *C. fujitertius* Sasa, and *C. echizensis* Sasa, but they also differ from the present species in the shape of dorsal appendage and gonostylus (cf. Sasa and Kikuchi, 1995).

6. *Chironomus mongolfegeus* sp. nov. (Figs. 6 a-d)

A male was collected in a tent, in Gobi Desert, on August 10, 1995. Holotype: No. 306:70.

Male. BL 7.44 mm, WL 3.40mm, WW/WL 0.29. Ground color of scutum yellow, stripes brown, scutellum yellow, postnotum brown, legs almost uniformly brownish yellow, abdominal tergites also largely brownish yellow and caudal margins of II, III, IV, VII and VIII are yellow. Frontal tubercles (Fig. 6 a) prominent, almost cylindrical, 40 μm long, 16 μm in diameter, and 39 μm apart from each other. Eyes bare, ER 0.36. Antenna with 11 flagellar segments, AR 3.83, AHR 0.57. Palp long, P/H 1.15. SO 28:30, CL 22. Antepronotum narrowly united in the middle, PN 0:0. The numbers of setae on scutum and scutellum are DM 18, DL 30:30, PA 8:8, and SC 30. Wing bare, SQ 18, RR 0.27, VR 1.01, R/Cu 1.14. Front tarsi both lost, mLR 0.61, hLR 0.71, mBR 3.1, hBR 4.2. Pulvilli well developed.

Hypopygium in Fig. 6 b. Anal point more stout than in the next species, widest at base, and slightly constricted in the middle. Dorsal appendage (Fig. 6 c) wider and more strongly curved, ventral appendage (Fig. 6 d) also wider than in the next species, widest at base and nearly straight, with 28 recurved setae and 5 short caudally directed setae. Gonostylus slender, inner margin concave, lateral margin abruptly constricted near apex.

Remarks. This species is similar in basic structure to the next species, but can be differentiated by the structure of hypopygium, as shown in the key. It is somewhat related to *C. salinarius* Kieffer, a cosmopolitan species breeding in brackish swamps, in that anal point is constricted in the middle and dorsal appendage is smoothly curved, but differs from the latter in that body coloration is paler, anal point is stout, dorsal appendage is wider, and gonostylus is strongly constricted near apex (ref. Sasa and Kikuchi, 1995).

7. *Chironomus mongolgeheus* sp. nov. (Figs. 7 a-d)

Thirty seven males were collected at Bogd (# 1) on August 13, 1996. Holotype: No. 308:61. Paratypes: other 36 males, No. 308:62; 310:01-35.

Male. BL 9.96-11.12 (10.35 in average of 8) mm, WL 4.80-5.02 (mean 4.87) mm (both larger than in most other species of *Chironomus*), WW/WL 0.26-0.29 (0.27). Ground color of scutum yellow, stripes and postnotum brown, scutellum yellow, abdominal tergites almost uniformly brownish yellow; femora and tibiae uniformly yellow, tarsi I, II and III largely yellow and each with an apical dark ring, IV yellow for basal half and brown for distal half, V brown.

Frontal tubercles (Fig. 7 a) prominent, 68 μm long, 28 μm wide and 40 μm apart from each other in the holotype. Eyes bare, ER 0.15-0.29 (0.21). Antenna with 11 flagellar segments, AR 4.45-5.41 (4.74, very high), AHR 0.56-0.63 (0.60). Palp relatively short, P/H 0.93-0.98 (0.96). SO 38-58 (44.3), CL 36-50 (41.8). Antepronotum narrowly united in the middle, without lateral setae. DM 18-24 (20.7), DL 36-42 (40.3), PA 5-8 (6.8), SC 36-44 (41.2). Wing bare, SQ 14-28 (22.3), RR 0.36-0.50 (0.44), VR 1.05-1.11 (1.08), R/Cu 1.12-1.17 (1.15). fLR 1.26-1.37 (1.32, relatively small), mLR 0.57-0.59 (0.58), hLR 0.68-0.71 (0.70), fTR 0.20-0.22 (0.21), fBR 4.3-7.3 (5.8, very high), mBR 2.7-4.0 (3.2), hBR 3.0-4.9 (3.9). Pulvilli well developed.

Hypopygium in Fig. 7 b. Anal point long, narrow, slightly constricted in the middle, and darkly pigmented, or narrow and sickle-shaped in lateral view. Dorsal appendage (Fig. 7 c) composed of a long and low base bearing 6-8 inner setae, and a narrow, straight and parallel-sided distal horn, which is slightly curved inwards at distal 1/5. Ventral appendage (Fig. 7 d) long, narrow, slightly curved and bearing some 30 recurved setae on distal 1/4. Gonostylus narrow, inner margin concave, and lateral margin constricted near apex.

Remarks. This species is somewhat similar among the European species to *C. annularis* (Degeer) in that anal point is slender, frontal tubercles well developed, tarsi with long beards, and body coloration is not black but largely brown, but differs from the latter in that

drosal appendage is stout and more strongly curved, ventral appendage is curved, and gonostylus is wider and constricted near apex. It is most closely related among the Japanese species of this genus to *C. fujiprimus* Sasa, since anal point is narrow, antepronotum without lateral setae, dorsal appendage is horn-like, scutal stripes and abdominal tergites almost uniform in color, and frontal tubercles are well developed, but in the latter body coloration is largely greenish yellow, AR is smaller (3.2-3.9), fLR larger (1.41-1.53), fBR smaller (1.9-2.4), anal point is constricted in the middle, and gonostylus is narrower and not constricted near apex (ref. Sasa and Kikuchi, 1995).

8. *Chironomus mongolheius* sp. nov. (Figs. 8 a-d)

Thirty six males were collected at Bogd (# 4), 1,500 m high from sea level, on August 13, 1996. Holotype: No. 308:63. Paratypes: No. 308:64-71, 310:38-63.

Male. BL 6.28-9.86 (8.32 in average of 6) mm, WL 2.88-4.22 (3.63) mm, WW/WL 0.26-0.28 (0.27). Ground color of scutum yellow, stripes brown (dark brown in some specimens), scutellum yellow, postnotum dark brown, leg segments almost uniformly brownish yellow (tarsi V darker) and without dark rings, abdominal tergites almost uniformly brown (tergites VII and VIII with pale area along caudal margin). Frontal tubercles (Fig. 8 a) prominent, 38 μm long, 11 μm in diameter, and 34 μm apart from each other. Eyes bare, ER 0.13-0.29 (0.23). Antenna with 11 flagellar segments, AR 3.98-4.75 (4.35), AHR 0.56-0.67 (0.60). Palp relatively short, P/H 0.83-1.00 (0.92). SO 42-64 (48.8), CL 36-70 (47.5). DM 24-44 (34.2), DL 24-46 (32.0), PA 8-16 (10.7), SC 32-52 (40.0).

Wing bare, R-M area dark, SQ 18-24 (20.7, relatively small), RR 0.28-0.40 (0.33), VR 1.03-1.07 (1.05), R/Cu 1.13-1.16 (1.14). fLR 1.25-1.46 (1.37), mLR 0.49-0.57 (0.54), hLR 0.62-0.68 (0.65), fTR 0.23-0.28 (0.25), fBR 3.3-7.8 (5.3), mBR 2.8-3.8 (3.3), hBR 3.4-4.8 (4.2).

Hypopygium in Fig. 8 b. Anal point relatively short, narrow and constricted in the middle. Dorsal appendage (Fig. 8 c) composed of a high base with rounded margin and bearing 6-8 inner setae, and a distal horn which is largely straight and abruptly hooked apically. Ventral appendage (Fig. 8 d) relatively short, straight and almost parallel-sided, bearing 32 short, recurved setae and 6 caudally directed short setae in the holotype. Gonostylus with nearly straight inner margin and smoothly rounded lateral margin, not constricted near apex.

Remarks. This species is most closely related among the European species to *C. plumosus* (Linnaeus) according to the key of Pinder (1978), in that anal point is narrow and constricted at base, dorsal appendage is not shoe-form, tarsi with long beards, and body is not entirely black, but the latter is a very large species breeding in lakes throughout the world, abdomen with conspicuous marks, and dorsal appendage is band-form. It is most closely related among the Japanese species also to *C. fujiprimus* Sasa, but also differs from the latter in body coloration and in the shape of dorsal appendage and gonostylus.

9. *Dicrotendipes nervosus* (Staeger, 1893) (Figs. 9 a-e)

Twelve males were collected at Bogd on August 13, 1996 (# 7), at about 1,500 m high from sea level. No. 308: 81, 82, 310:81-85, 311:39-43. In the first two specimens measured, BL 5.22, 5.10 mm, WL 2.64, 2.46 mm, WW/WL 0.27, 0.28. Body almost entirely yellow, scutal stripes, postnotum, distal half of front tibia, all tarsal segments and hypopygium slighly brownish. ER 0.24, 0.23, AR 3.00, 2.95, AR 0.67, 0.63, P/H 1.26, 1.36, PN all 0, DM 21, 18, DL 14:16, 15:16, PA 6:7, 5:6, SC 13,16. Wing bare, SQ 18:18, 11:13, RR 0.21, 0.20, VR 1.02, 1.07, R/Cu 1.12, 1.11. fLR 1.58, 1.64, mLR 0.52, 0.53, hLR 0.67, 0.68, fTR 0.23, 0.24, fBR 2.7, 2.8, mBR 3.7, 3.2, hBR 5.9, 3.5. Frontal tubercles in Fig. 9 a. Antepronotum (Fig. 9 b) narrowly united in the middle, without lateral setae. Hypopygium in Fig. 9 c. Both dorsal appendage (Fig. 9 d) and ventral appendage (Fig. 9 e) extremely long, gonostylus also very long, narrow and smoothly curved.

Remarks. The above measurement data and the structure are almost coincident with those obtained with specimens of *D. nervosus* collected in Europe (Pinder, 1978, Fig. 157 D) and Japan (Sasa and Kikuchi, 1995), but the shape of appendages and gonostylus seems to be slightly different from those of the above reports.

10. *Cryptotendipes mongolijeus* sp. nov. (Figs. 10 a-j)

Three males were collected at Bogd on August 13, 1996 (# 9). Holotype: No. 310:87. Paratypes: No. 310: 88, 89.

Male. BL 4.88, 4.70, 3.92 mm, WL 2.14, 2.16, 1.80 mm, WW/WL 0.31, 0.31, 0.29. Ground color of scutum pale, stripes dark brown, scutellum pale, postnotum dark brown, legs and abdominal tergites brownish yellow. Head in Fig. 10 a. Frontal tubercles absent. Eyes bare, ER 0.44, 0.32, 0.33. Antenna with 13 flagellar segments, AR 2.97, 2.71, 2.29. Palp nearly as long as the width of head, P/H 0.91, 0.96, 1.05. SO 10:10, 12:12, 12:12, CL 14, 16, 12. Antepronotum (Fig. 10 b) united in the

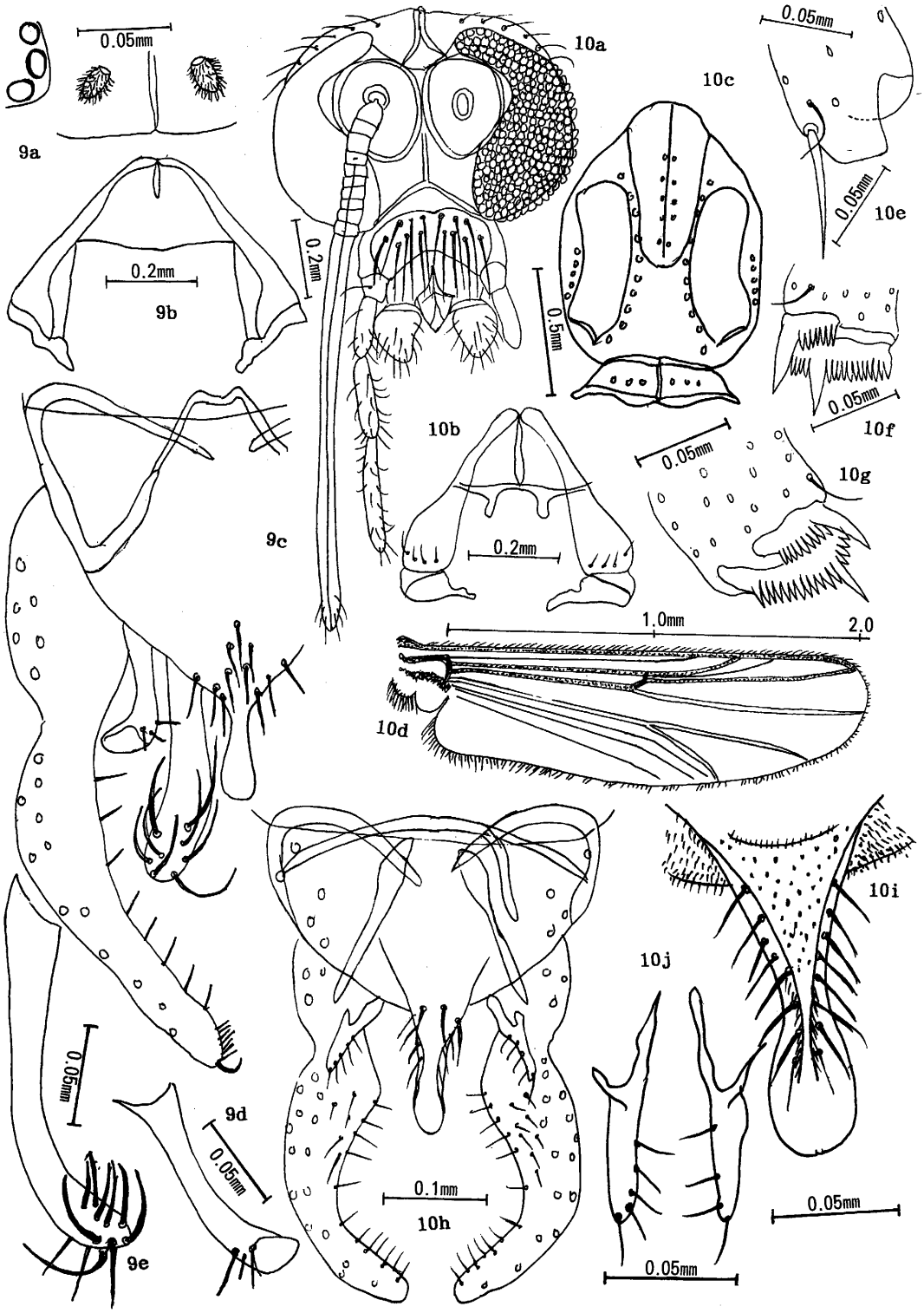

Fig. 9 ***Dicrotendipes nervosus*** **(Staeger, 1893)** a: frontal tubercles; b: antepronotum; c: hypopygium; d: left dorsal appendage; e: left ventral appendage. **Fig. 10** ***Cryptotendipes mongolijeus*** **sp. nov.** a: head; b: antepronotum; c: scutum and scutellum; d: wing; e: tip of front tibia; f: tip of middle tibia; g: tip of hind tibia; h: hypopygium; i: anal point; j : dorsal appendages.

middle, with 5:5, 4:4, 2:2 lateral setae. Distribution of setae on scutum and scutellum in Fig. 10 c. DM 10, 8, 4, arising from some distance from the anterior margin of scutum. DL 10:10, 12:12, 8:8. SC 6, 6, 10.

Wing bare, finely granular, venation as in Fig. 10 d. SQ 21:22, 20:20, 24:24. Anal lobe slightly produced inwards. RR 0.37, 0.36, 0.35, VR 1.11, 1.20, 1.11, R/Cu 1.07, 1.06, 1.04. Tip of front tibia (Fig. 10 e) with a broad and rounded scale. Tips of middle and hind tibiae (Figs. 10 f, g) with two comb scales, both with a spur. fLR 1.36, 1.34, 1.44, mLR 0.45, 0.49, 0.45, hLR 0.69, 0.65, 0.62, fTR 0.25, 0.25, 0.2, fBR 3.4, 3.6, mBR 3.5, 3.2, hBR 3.6, 3.6. Pulvilli well developed, brush-like.

Hypopygium in Fig. 10 h. Ninth tergite with two short setae at the base of anal point, otherwise without setae in the median portion. Anal point (also in Fig. 10 i, ventral view) constricted in the middle and apically rounded, with some 16 short setae arising from the ventral and lateral portions. Dorsal appendages (also in Fig. 10 j) rather small, horn-like, narrow and straight, with V-shaped base and 4 or 5 setae on inner margin and apex. Ventral appendage absent. Gonostylus fused with gonocoxite, inner margin strongly produced inwards near the base and conspicuously concave towards rounded apex, with 12-14 short setae along inner margin.

Remarks. This species is a typical member of the *Harnischia* complex of the tribe Chinonomini, since antenna with 11 flagellar segments, apex of middle and hind tibiae with two comb scales each with a spur, dorsal appendage is reduced and ventral appendage is absent, and seems to be belonging to the genus *Cryptotendipes* Lenz, 1941, as its dorsal appendage is rod-*like, with a few short setae but without microtrichia. Reviews of this genus were made by Saether (1977) and Cranston et al.* (1989a), and the present species seems to be closest to a Nearctic species, *C. emorosus* (Townes, 1945), in that ninth tergite without dorsal hump, inner margin of gonostylus with conspicuous median projection that forms distinctive concavity in the apical half, anal point is well developed, and dorsal appendage without microtrichia. However, in the original description of this species by Townes (1945, p. 161, Fig. 185) by the generic name of *Harnischia*, anal point is not constricted at base, and gonostylus is much longer and narrower, and seems to be quite different from the present species. The present species also differs from the two species of this genus recorded from Japan, *C. oyabeprimus* Sasa, Kawai et Ueno, 1988 and *C. tamacutus* Sasa, 1983, in the shape of gonostylus and anal point. The present species is also unusual as a member of this genus in that setae on dorsal appendage are arising from not only the apical portion, but are distributed to the distal half of the inner margin.

***Mongolchironomus* gen. nov.**

A new genus belonging to the *Harnischia* complex of the tribe Chironomini. Antenna with 11 flagellar segmets. Antepronotum with lateral setae (a character in common to members of the *Harnischia* complex). Gonostylus fused with gonocoxite, long, slender, expanded medially near the base, and inner margin is concave towards rounded apex. Ventral appendage absent. Dorsal appendage situated in the basal portion of gonocoxite, thumb-like, parallel-sided and apically rounded, bearing 3 to 5 setae and clothed with microtrichia except on the lateral portion, and directed laterally (not medially like in most other species of this complex). Containing only the following two species collected this time in Mongolia.

The species of this genus are similar in the structure of hypopygium to *Microchironomus* Kieffer, 1918, in that dorsal appendage of gonocoxite is slender, rod-like and bearing a few setae, ventral appendage is absent, and gonostylus is expanded basally, but differs from it in that dorsal appendage is broad, covered by microtrichia and directed laterally, and gonostylus without apical hook. Genotype: *M. mongoljekeus* sp. nov.

11. *Mongolchironomus mongoljekeus* sp. nov.

(Figs. 11 a-m)

Altogether 14 males were collected at Bogd on August 13, 1996. Holotype: No. 308:84. Paratypes: other 13 males, No. 308:85, 310:91-97, 311:44-48.

Male. BL 4.10-4.66 (4.30 in average of 8) mm, WL 1.70-1.96 (1.80 in average of 14)mm, WW/WL 0.28-0.31 (0.29). Median and lateral stripes dark brown and clearly differentiated from the large yellow humeral and the median posterior areas of scutum; scutellum yellow, postnotum dark brown; legs and abdominal tergites largely yellow. Head in Fig. 11 a. Eyes bare, ER 0.28-0.42 (0.35). Palp long, P/H 1.02-1.15 (1.08). Frontal tubercles (Fig. 11 b) very small, semicircular, 13 μm wide, 11 μm high and 90 μm apart from each other. AR 2.26-2.66 (2.41 in average of 14), AHR 0.55-0.65 (0.60). Palp relatively long, P/H 1.02-1.15 (1.08). SO 12-20 (14.6), CL 10-24 (14.1). Antepronotum (Fig. 11 c) united in the middle, with 3-5 (4.0) lateral setae, and the bridge connecting the two lobes without processes such as seen in the next species. DM 3-6 (4.7), DL all 8, PA 3-6 (4.1), SC 6-9 (8.0).

Wing in Fig. 11 d. Squama with 12-18 (14.7) fringe

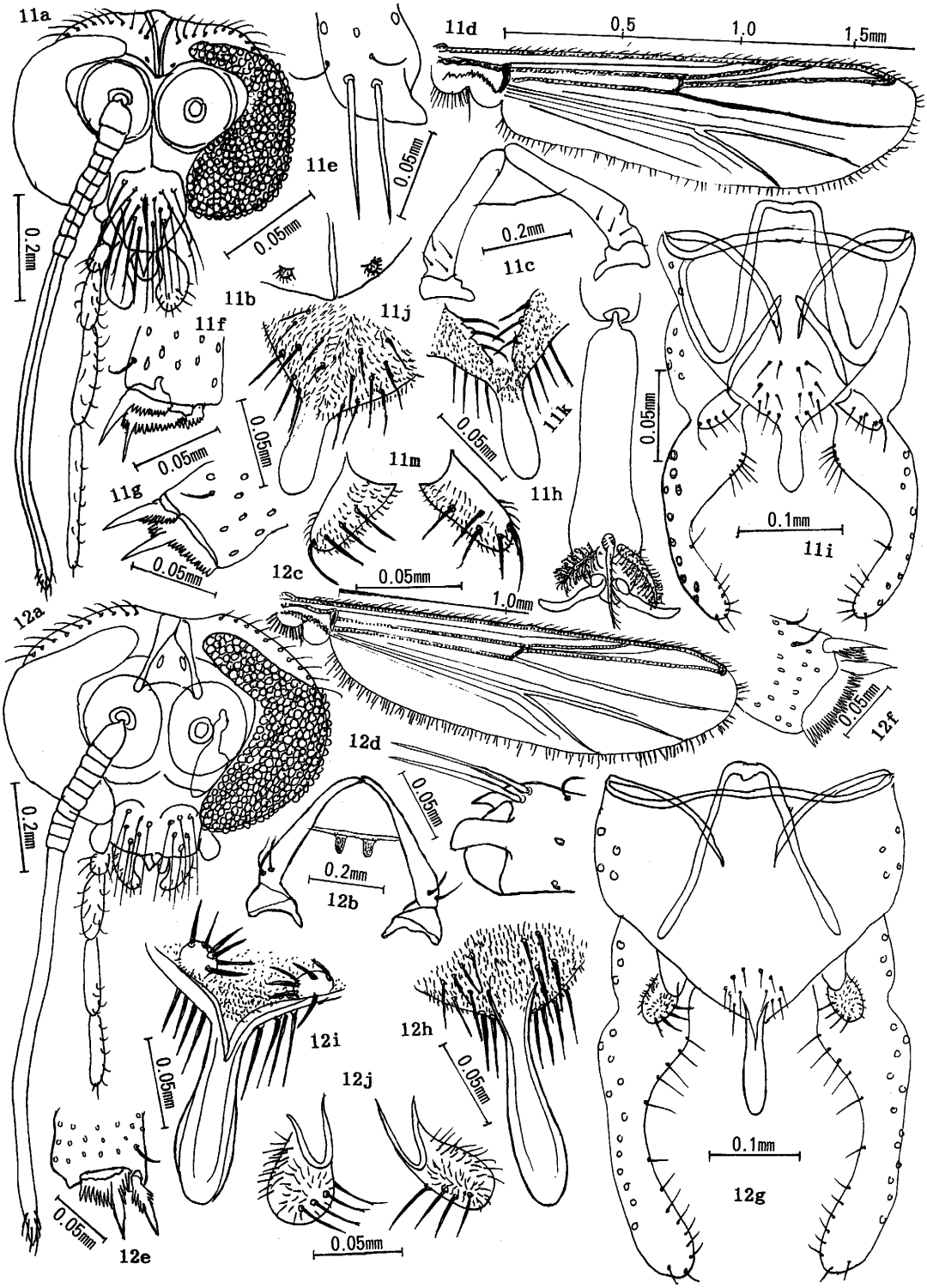

Fig. 11 ***Mongolchironomus mongoljekeus*** **sp. nov.** a: head; b: frontal tubercles; c: antepronotum; d: wing; e: tip of front tibia; f: tip of middle tibia; g: tip of hind tibia; h: front tarsus V; i: hypopygium; j: anal point, dorsal view; k: anal point, ventral view; m: dorsal appendages. **Fig. 12** ***Mongolchironomus mongolkeleus*** **sp. nov.** a: head; b: antepronotum; c: wing; d: tip of front tibia; e: tip of middle tibia; f: tip of hind tibia; g: hypopygium; h: anal point, dorsal view; i: anal point, ventral view; j: dorsal appendages.

hairs, anal lobe obtuse. RR 0.32-0.42 (0.37), VR 1.06-1.15 (1.12), R/Cu 1.06-1.09 (1.07). Tip of front tibia (Fig. 11 e) with a broad and somewhat sickle-shaped process. Tips of middle and hind tibiae (Figs. 11 f, g) with two comb scales, both with a short spur. fLR 1.32-1.44 (1.38), mLR 0.45-0.51 (0.47; very small), hLR 0.63-0.67 (0.65), fTR 0.21-0.24 (0.23), fBR 3.3-6.4 (5.1), mBR 2.4-3.2 (2.9), hBR 4.0-7.0 (5.4). Tips of tarsi V with a pair of simple claws, an empodium, and a pair of brush-like pulvilli (Fig. 11 h, front tarsus V).

Hypopygium in Fig. 11 i. Bands of ninth tergite separated in the middle. Anal point (Fig. 11 j, dorsal; Fig. 11 k, ventral view) slightly constricted at base, with some 16 short setae at its base on dorsal side, and a median V-shaped groove with straight edge bearing 3 pairs of setae on ventral side. Gonocoxite each with a finger-like, laterally directed dorsal appendage (Fig. 11 m) bearing a long and curved apical seta and 3 or 4 inner setae, and clothed with microtrichia. Gonostylus widest at base and constricted in the middle, distal half abruptly curved inwards, bearing 6 setae on the basal expanded portion of inner margin.

Remarks. This species can be distinguished from the previously known species of the *Harnischia* complex by the characters stated in the definition of this new genus.

12. *Mongolchironomus mongolkeleus* sp. nov.

(Figs. 12 a-j)

Three males were collected at Bogd (# 9) on August 13, 1996. Holotype: No. 308:86. Paratypes: No. 308:87, 88.

Male. BL 5.46, 5.36, 5.44 mm, WL 2.40, 2.18, 2.10 mm (all larger than in the preceding species), WW/WL 0.28, 0.30, 0.30. Scutal stripes black and clearly differentiated from the large yellow humeral and median posterior areas, scutellum yellow, postnotum black, legs and abdominal tergites brownish yellow. Head in Fig. 12 a. ER 0.31, 0.26, 0.32. Frontal tubercles very small, crescent-shaped, 10 μm wide and 8 μm high. AR 3.11, 3.03, 3.00 (larger than in the preceding species), AHR 0.65, 0.70, 0.62. Palp shorter than in the preceding species, P/H 0.81, 0.79, 1.00. SO 16-20 (17.7; larger), CL 11, 10, 10. Antepronotum (Fig. 12 b) connected in the middle, with 2:2, 4:4, 4:4 lateral setae, the bridge connecting the two lobes bears a pair of long, darkly pigmented processes which are absent in the preceding species. DM only 3, 3, 0, DL 12-15 (13.8), PA 5 or 6 (5.5), SC 14-17 (15.7; much larger).

Wing bare, venation in Fig. 12 c. Squama with 12-18 (15.0) fringe hairs in the holotype. RR 0.42, 0.33, 0.36, VR 1.08, 1.13, 1.11, R/Cu 1.05, 1.08, 1.06. Tip of front tibia (Fig. 12 d) with 3 processes, a narrow and sharply pointed one, a broad and rounded one, and a long, somewhat quadrangular one. Tips of middle and hind tibiae (Figs. 12 e, f) with a narrow and a broad comb scales, both with a spur. fLR 1.41, 1.45, 1.44, mLR 0.53, 0.53, 0.51, hLR 0.68, 0.68, 0.66, fTR 0.26, 0.27, 0.25, fBR 6.3, 4.2, 5.2, mBR 5.3, 5.3, 5.1, hBR 5.7, 4.9, 4.3. Pulvilli pad-like and covered with brush.

Hypopygium in Fig. 12 g. Anal point (Fig. 12 h, dorsal; Fig. 12 i, ventral view) is more conspicuously constricted at base than in the preceding species, the V-shaped groove on the ventral side (Fig. 12 i) has a pair of rounded processes bearing 6 strong setae. Dorsal appendages (Fig. 12 j) are shorter and slightly expanded, not conical as in the preceding species, and bear 4 setae on inner margin but without apical seta. Gonostylus fused with gonocoxite, widest at base, abruptly curved and constricted in the middle, the setae on inner margin are distributed more widely than in the preceding species, not concentrated on the basal convex portion.

Remarks. These specimens are quite similar in the basic structure to the preceding species, and were considered first as its larger form, but is described as a different species belonging to the same new genus, since BL, WL, and AR are much larger, palpi are shorter than the width of head and P/H is conspicuously smaller than 1.0, and the numbers of scutellar setae and the values of LR are larger. The shape of dorsal appendage is different between the two groups, the base is U-shaped and expanded towards middle in this species (Fig. 12 j), while in the former the base is only slightly concave and tapering towards apex (Fig. 11 m). Anal point is longer, narrower, constricted at base and with lateral ridges in this species (Fig. 12 h), while it is stouter, parallel-sided and without lateral ridge in the former (Fig. 11 j). The groove on the ventral side of the base of anal point bears a pair of rounded process bearing 5 or 6 setae in this species (Fig. 12 i), while they are absent in the former (Fig. 11 k). The bridge connecting antepronotum bears a pair of large dark processes in this species (Fig. 12 b), while they are absent in the former (Fig. 11 c).

13. *Polypedilum mongollemeus* sp. nov.

(Figs. 17 a-k)

A male was collected at River Orkhon (# 35), 1,775 m high, on August 18, 1996. Holotype: 308:89.

Male. BL 4.08 mm, WL 2.32 mm, WW/WL 0.30. Ground color of scutum brown, stripes dark brown, scutellum brown, leg segments uniformly brown, abdom-

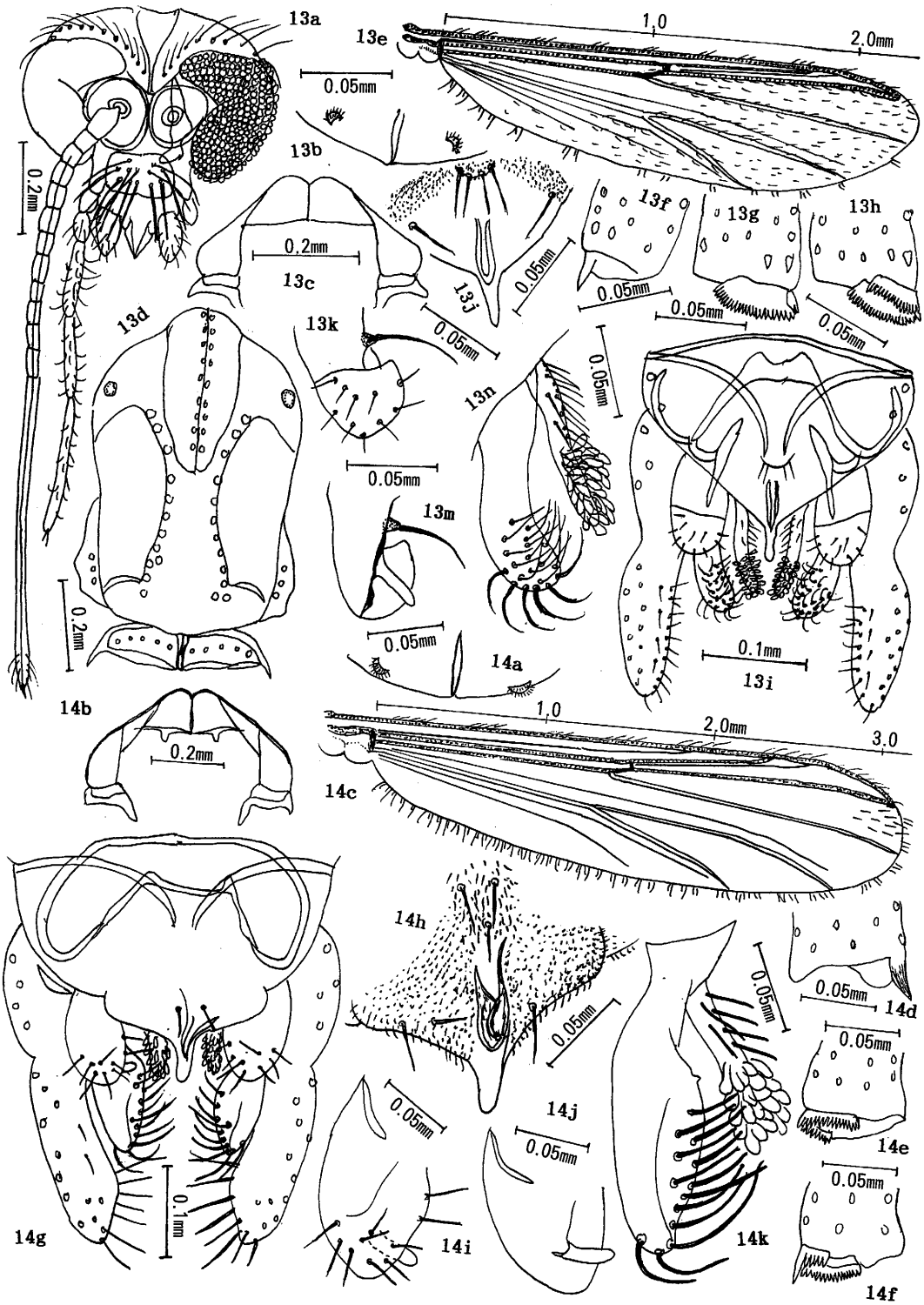

Fig. 13 ***Micropsectra junci*** **(Meigen, 1818)** a: head; b: frontal tubercles; c: antepronotum; d: scutum and scutellum; e: wing; f: tip of front tibia; g: tip of middle tibia; h: tip of hind tibia; i: hypopygium; j: anal point; k: dorsal appendage, dorsal view; m: dorsal appendage and digitus, ventral view; n: median and ventral appendages.

Fig. 14 ***Micropsectra mongolmeneus*** **sp. nov.** a: frontal tubercles; b: antepronotum; c: wing; d: tip of front tibia; e: tip of middle tibia; f: tip of hind tibia ; g: hypopygium; h: anal point; i: dorsal appendage, dorsal view; j: dorsal appendage and digitus, ventral view; k: median and ventral appendages.

inal tergites almost uniformly brown, tergites VII and VIII each with a narrow pale area along caudal margin. Head in Fig. 17 a. Frontal tubercles absent. Eyes bare, ER 0.45. Antenna with 13 flagellar segments, AR 1.63, AHR 0.56. Palp long, P/H 1.27. SO 9:10, CL 12. Antepronotum (Fig. 17 b) separated in the middle with a V-shaped groove, without lateral setae. Distribution of setae on scutum and scutellum in Fig. 17 c. DM 16, DL 15:15, PA 5:5, SC 12.

Wing membrane bare, bluish and finely granular, venation in Fig. 17 d. SQ 21:22, RR 0.17, VR 1.10, R/Cu 1.11. Anal lobe nearly rectangular. Tip of front tibia (Fig. 17 e) with a rounded scale bearing a long and stout seta. Tip of middle and hind tibiae (Figs. 17 f, g) with a narrow and a broad comb scales, the former with a long spur and the latter without spur. fLR 1.23 (vary small), mLR 0.58, fTR 0.23, fBR 3.0, mBR 3.5 (hind tarsi lost). Pulvilli well developed, brush-like.

Hypopygium in Fig. 17 h. Anal point long, slender and almost parallel-sided. Ninth tergite with 6 long setae in the middle portion, and 6 setae on both sides of posterior margin flanking anal point. Dorsal appendage (Fig. 17 i, right) roughly C-shaped, inner margin of basal portion very high and strongly concave, with 3 inner setae, and looks quite differently when seen from the frontal angle (Fig. 17 j, left). Ventral appendage (Fig. 17 k) long, slender and parallel-sided, with 8 recurved setae and a long apical seta. Gonostylus slightly expanded in the middle, with an apical seta and 12 long setae along inner margin.

Remarks. This species is a typical member of the *nubeculosum* group of subgenus *Polypedilum* s. str., since dorsal appendage composed of a broad base bearing inner setae, and a distal horn bearing a long lateral seta. In the key to this group compiled by Sasa and Kikuchi (1995), it is closest to *P. kunigamiense* Sasa et Hasegawa, 1988, in that body with brown and yellow colorations, antepronotum without setae, abdominal tergites are almost uniformly brown, AR is larger than 1.4 and smaller than 1.7, frontal tubercles are absent, and lateral seta on dorsal appendage arises from about middle of the distal horn, but differs essentially in the shape of dorsal appendage (basal portion is strongly elevated and distal horn is more strongly curved in the present species), and fLR is much smaller (1.85-1.93 in *P. kunigamiense,* after Sasa and Hasegawa, 1988).

14. *Micropsectra junci* (Meigen, 1818)

(Figs. 13 a-n)

Three males were collected at the side of River Orkhon on August 18, 1996 (No. 308:91-93). BL 4.10, 3.82, 3.10 mm, WL 2.32, 2.26, 2.36 mm, WW/WL 0.28, 0.27, 0.29. Scutal stripes and postnotum brown, other body portions largely yellow. Head in Fig. 13 a. Eyes bare, ER 0.39, 0.28, 0.30. Frontal tubercles (Fig. 13 b) very small, 4 μm wide and 5 μm high, 22 μm apart from each other. Antenna with 13 flagellar segments, AR 1.14, 1.14, 1.11, AHR 0.51, 0.48, 0.48. Palp very long, P/H 1.41, 1.36, 1.37. SO 12:12, 12:14, 14:14, CL 16, 18, 18. Antepronotum (Fig. 13 c) widely separated in the middle, PN all 0. Distribution of setae on scutum and scutellum in Fig. 13 d. DM 13, 12, 23, DL 11:12, 11:11, 11:13, SC 12 (scutellum is lost in 2 specimens).

Wing (Fig. 13 e) with rather small numbers of macrotrichia on the distal half and on the principal veins. SQ all 0, RR 0.36, 0.35, VR 1.14, 1.11, 1.14, R/Cu 1.10, 1.10. Terminal process of front tibia (Fig. 13 f) short, narrow and sharply pointed. Terminal comb scale of middle and hind tibiae (Figs. 13 g, h) contiguous and without spur. fLR 1.45, 1.54, mLR 0.54, 0.69, fTR 0.24, 0.22, fBR 4.2, mBR 5.8 (hind tarsi all lost). Pulvilli absent.

Hypopygium in Fig. 13 i. Anal point (also in Fig. 13 j) small, narrow and parallel-sided, with a U-shaped basal ridge, and a small tubercle bearing 4 short setae on its base. Dorsal appendage (Fig. 13 k) semicircular, 34 μm high and 40 μm wide in the holotype, length/width ratio 0.85, 0.90, 1.00, with 10 short setae on dorsal side and a long basal seta arising from a large tubercle. Digitus and ventral aspect of dorsal appendage in Fig. 13 m, Di/DA ratio 0.75, 0.74, 0.78. Median and ventral appendages in Fig. 13 n. The former relatively long, narrow and nearly straight, bearing some 10 simple setae on basal half and some 20 short spoon-like setae on distal half, MA/VA length ratio 0.91, 0.96, 0.93. Ventral appendage finger-like, bearing some 20 short, recurved setae on dorsal side and 4 caudally directed setfae on ventral side of the distal portion. Gonostylus fused with gonocoxite, slightly expanded in the middle, with 12 short setae in two rows on inner margin.

Remarks. This species is structurally a typical member of the genus *Micropsectra* Kieffer, 1909, and is provisionally identified as *M. junci* (Meigen, 1818), since the structures are almost coincident with those described by European workers, especially by Pinder (1978, p. 144, Fig. 175A), in that combs of middle and hind tibiae are fused and without spur, scutal stripes are distinct, AR is about 1.1, anal point is narrow and triangular, and median appendage is relatively long and bearing spoon-shaped setae. This species has also been recorded from Lake Towada, northern Honshu, by Sasa (1991, p. 69).

15. *Micropsectra mongolmeneus* sp. nov.

(Figs. 14 a-k)

A male was collected at the side of the River Orkhon on August 18, 1996. Holotype: 308:94.

Male. BL 5.80 mm, WL 3.18 mm, WW/WL 0.26 (very narrow). Body almost entirely black. Frontal tubercles (Fig. 14 a) small, cup-like, 9 μm wide, 5 μm high, and 77 μm apart from each other. Eyes bare, ER 0.39. Antenna with 13 flagellar segments, AR 2.69, AHR 0.66. Palp long, P/H 1.37. SO 16:16, CL 20. Antepronotum (Fig. 14 b) widely separated in the middle, without lateral setae. DM 6, DL 12:14, PA 3:4, SC 10.

Wing (Fig. 14 c) with only about 10 macrotrichia in the extreme tip area (quite unusual character as a member of this genus). Squama bare, anal lobe nearly flat. RR 0.24, VR 1.01, R/Cu 1.07. Tip of front tibia (Fig. 14 d) with a short, narrow and apically pointed spur. Terminal comb scales of middle tibia (Fig. 14 e) contiguous, very low and without spur. Terminal comb scales of hind tibia (Fig. 14 f) also very low and contiguous, but with one short spur. fLR 1.15 (very small), mLR 0.46 (unusually small), hLR 0.62, fTR 0.20, fBR 6.5, mBR 4.0, hBR 3.7. Pulvilli absent.

Hypopygium in Fig. 14 g. Bands of ninth tergite separated. Anal point (Fig. 16 h) very small, widest at base and apically rounded, with a few short setae near its base. Dorsal appendage roughly semicircular, 62 μm wide and 122 μm long, and with 10 short setae on dorsal side, basal seta absent (Figs. 14 i,j). Digitus short, 32 μm long and 8 μm wide, Di/DA 0.52. Median and ventral appendages in Fig. 14 k. The former 92 μm long, composed of a straight shaft bearing 6 or 8 setae, and a distal expanded portion bearing 30 short, spoon-like setae. Ventral appendage 184 μm long (MA/VA 0.50), conspicuously expanded at about middle, bearing 20 recurved setae on inner side of distal half. Gonostylus long, parallel-sided, bearing 8 long setae on inner margin of apical portion.

Remarks. This specimen was collected at the same site as in the preceding species, and also belongs to the genus *Micropsectra,* but obviously represents a different species, since body is much larger, entirely black, wing with only a few macrotrichia restricted to the tip area, AR is much larger, VR is nearly 1.0 and smaller, fLR and mLR is much smaller, and median appendage is much shorter. Among the European species of this genus, it is somewhat related to *M. notescens* (Walker) in that AR is high, body is large and entirely black, median appendage is relatively short and with spoon-like setae, but the latter differs from the present species especially in that mLR is larger than 0.5, anal point and dorsal appendage are narrower, median appendage is shorter, ventral appendage is parallel-sided and not expanded in the middle. Among the species recorded from Japan, it is most closely related to *M. yunoprima* Sasa, 1984, collected in large numbers from the mountain lake of Yunoko, Nikko National Park, but the latter is still larger in body size and AR, wing with more numerous macrotrichia on larger area, ventral appendage is not medially expanded, median appendage is shorter and with smaller numbers of longer and narrower spoon-like setae (Sasa, 1984).

16. *Tanytarsus mongolneous* sp. nov. (Fig. 15 a-k)

Three males were collected at Bogd on August 13, 1996. Holotype: No. 308:96. Paratypes: 308:97, 98.

Male. BL 2.72, 2.66, 2.68 mm, WL 1.40, 1.32, 1.26 mm, WW/WL 0.28, 0.27, 0.29 mm. Ground color of scutum yellow, stripes, scutellum, postnotum and abdominal tergites brown, legs brownish yellow. Head in Fig. 15 a. Eyes bare, inner margin concave but without dorsomedial extension, ER 1.32, 1.26, 1.50. Frontal tubercles (Fig. 15 b) prominent, widest at base and apically rounded, 16 μm long, 7 μm in diameter, and 32 μm apart from each other. Antenna with 13 flagellar segments, AR 1.06, 1.04, 1.05, AHR 0.53, 0.53, 0.51. Palp about as long as the width of head, P/H 1.08, 0.98, 0.82. SO 9:9, 10:10, 10:10, CL 8, 10, 14. Antepronotum (Fig. 15 c) widely separated in the middle, without lateral setae. DM 8, 7, 8, DL 6:7, 8:8, 6:6, PA all 1, SC 6, 5, 6.

Wing with relatively small numbers of macrotrichia on the distal portion and on the principal veins, venation in Fig. 15 d. Squama bare, anal lobe nearly flat. RR 0.41, 0.50, 0.49, VR 1.14, 1.19, 1.15, R/Cu 1.06, 1.07, 1.09. Tip of front tibia (Fig. 15 e) with a short, narrow and pointed spur. Tips of middle and hind tibiae (Figs. 15 f, g) with two narrow comb scales, both with a spur. fLR 1.54, 1.61, 1.55, mLR 0.54, 0.51, 0.50, hLR 0.63, 0.62, 0.63, fTR 0.28, 0.26, 0.28, fBR 4.2, 4.3, mBR 5.2, 4.1, hBR 8.4, 7.6. Pulvilli absent.

Hypopygium in Fig. 15 h. Bands of ninth tergite separated in the middle. Anal point (also in Fig. 15 i) triangular, sharply pointed apically, with a pair of lateral ridges and 4 spine clusters in two rows, with 12 short basal setae and 4 pairs of lateral setae. Dorsal appendage (Fig. 15 j) somewhat sickle-shaped, inner margin concave, with 6 short setae on dorsal surface, and with 3 basal setae arising on prominent tubercles. Digitus long and slender, entirely exposed on inner side of dorsal appendage. Median and ventral appendages in Fig. 15 k; the former very long and slender, longer than the latter, MA/VA 1.17, 1.39, 1.19, with both simple and

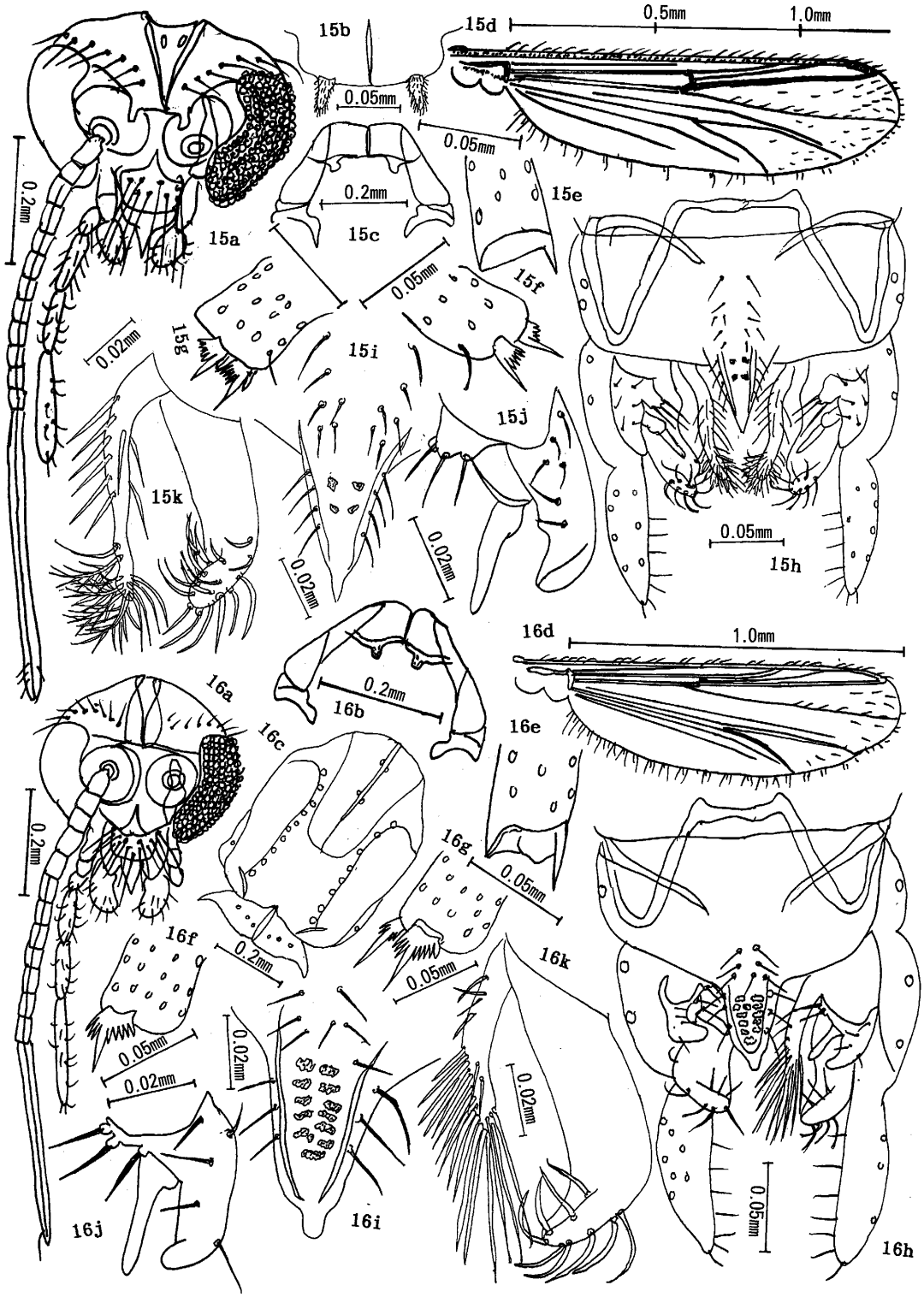

Fig. 15 ***Tanytarsus mongolneous*** **sp. nov.** a: head; b: frontal tubercles; c: antepronotum; d: wing; e: tip of front tibia; f: tip of middle tibia; g: tip of hind tibia; h: hypopygium; i: anal point; j: dorsal appendage and digitus; k: median and ventral appendages. **Fig. 16** ***Tanytarsus mongolopeus*** **sp. nov.** a: head; b: antepronotum; c: scutum and scutellum; d: wing; e: tip of front tibia; f: tip of middle tibia; g: tip of hind tibia; h: hypopygium; i: anal point; j: dorsal appendage and digitus; k: median and ventral appendages.

curved, and highly branched long and narrow setae arising on the distal portion, and some 10 simple setae arising on the median portion. Ventral appendage also long, slightly tapering towards rounded apex, with 18-20 recurved setae arising in the distal portion. Gonostylus with inner margin nearly straight, widest at about middle, and with 8 short setae on inner margin.

Remarks. This species is a member of the *yunosecundus* group of the genus *Tanytarsus* van der Wulp, 1874, since anal point with lateral ridges and spine clusters, digitus present and median appendage is longer than the ventral appendage (ref. Sasa and Kikuchi, 1995). However, it is quite unusual as a member of this group and differs remarkably from the previously known species in that anal point is triangular and sharply pointed apically, dorsal appendage with a large basal tubercle bearing 3 setae, and ventral appendage bears nume-rously branched apical setae.

17. *Tanytarsus mongolopeus* sp. nov. (Figs. 16 a-k)

A male was collected at Karakorum on August 18, 1996. Holotype: No. 308:99.

Male. BL 3.08 mm, WL 1.40 mm, WW/WL 0.31. Ground color of scutum yellow, stripes, scutellum and postnotum dark brown, legs and abdominal tergites brownish yellow. Head in Fig. 16 a. Eyes bare, reniform, ER 1.55. Frontal tubercles absent, a remarkable difference from the above species. Antenna with 13 flagellar segments, AR 0.81 (small), AHR 0.49. Palp long, P/H 1.08. SO 10:10. CL 14. Antepronotum (Fig. 16 b) widely separated, without lateral setae. Distribution of setae on scutum and scutellum in Fig. 16 c; DM only 4, DL 8:10, PA 1:1, SC 6.

Wing with very small numbers of macrotrichia only in the distal portion, venation in Fig. 16 d. Squama bare, anal lobe nearly flat. RR 0.49, VR 1.30 (larger), R/Cu 1.05. Tip of front tibia (Fig. 16 e) with a long and narrow spur. Tip of middle tibia (Fig. 16 f) with only one comb scale bearing a spur, tip of hind tibia (Fig. 16 g) with two narrow comb scales, both with a spur. fLR 1.86 (larger), mLR 0.55, hind tarsi lost. fTR 0.33. Tarsi V slender, pulvilli absent (Fig. 16 h, front tarsus V).

Hypopygium in Fig. 16 h. Bands of ninth tergite separated. Anal point (Fig. 18 i) widest at base and contricted near apex, with lateral ridges and 12 spine clusters in two rows, with 3 pairs of lateral setae and 8 short basal setae. Dorsal appendage (Fig. 16 j) longer than wide and sickle-shaped, with 5 dorsal and 3 basal inner setae. Digitus long, entirely exposed on inner side of dorsal appendage. Median and ventral appendages in Fig. 16 k. The shaft of the former about half as long as the latter but bearing very long and simple setae whose tips extending beyond tip of ventral sppendage. Ventral appendage finger-like but curved inwards apically, with only 8 recurved setae. Gonostylus widest at about middle and inner meargin nearly straight, with 8 setae on inner margin.

Remarks. This specimen also belongs to the *yunosecundus* group of the genus *Tanytarsus,* since anal point with lateral ridges and spine clusters, digitus long, and median appendage is very long and longer than the ventral appendage, but differs from the above species and also from all the previously known species of this group in that distal setae of median appendage are all very long and simple, anal point with as many as 12 spine clusters in two rows, and dorsal appendage is narrow and sickle-shaped (Sasa and Kikuchi, 1995).

18. *Cricotopus* (*Cricotopus*) *annulator* Goetghebuer, 1927 (Figs. 18 a-i)

Thirteen males were collected, 10 at Karakorum (# 28) on August 18, 1996 (No. 309:12, 18, 24-28; 311:66-71), and 3 at River Orkhon (# 38) on August 18, 1996 (No. 311:83-85).

Male. Eight males were measured. BL 2.86-3.86 (3.38 in average) mm, WL 1.58-2.00 (1.82) mm, WW/WL 0.31-0.33 (0.32). Ground color of scutum yellowish brown, stripes brown, humeral areas yellow; scutellum and postnotum dark brown, femora and tarsi largely brown, tibiae largely yellow and with short brown rings on both ends; dark and pale areas on abdominal tergites as in Fig. 18 g ; tergite I entirely white, oral half of II white and its distal half brown, III largely brown and with narrow pale areas along oral and caudal margins, IV with a pale area on caudal 1/3, V to VII with a pale area along caudal margin, VIII entirely brown, IX and hypopygium largely white.

Head in Fig. 18 a. Eyes pubescent, ER 0.59-0.84 (0.71). Antenna with 13 flagellar segments, AR 1.21-1.40 (1.32), AHR 0.52-0.56 (0.54). P/H 0.97-1.09 (1.03). SO 8-10 (8.8), CL 8-13 (11.0). Antepronotum (Fig. 18 b) united in the middle, with 3-6 (4.0) lateral setae. Distribution of setae on scutum and scutellum in Fig. 18 c. DM 16-24 (20.4), all minute. DL 18-26 (22.2), all minute, decumbent and arising from very small pits. PA 3-6 (4.0), SC 6-8 (6.8), both well developed. SQ 6-10 (8.2), RR 0.45-0.49 (0.47), VR 1.08-1.13 (1.11), R/Cu 1.04-1.10 (1.07). fLR 0.66-0.69 (0.67), mLR 0.49-0.51 (0.50), hLR 0.57-0.61 (0.59), fTR 0.12-0.13, fBR 0.18-0.23 (0.21), mBR 2.1-2.3 (2.2), hBR 2.2-2.4 (2.3). Pulvilli absent.

Distribution of setae on abdominal tergites in Fig. 18 g (No. 309:18), 8 on I, 14 on II, 16 on III, 18 on IV, 20

Fig. 17 ***Polypedilum mongollemeus*** **sp. nov.** a: head; b: antepronotum; c: scutum and scutellum; d: wing; e: tip of front tibia; f: tip of middle tibia; g: tip of hind tibia; h: hypopygium; i: dorsl appendage, dorsal view; j: dorsal appendage, ventral view; k: ventral appendage. **Fig. 18** ***Cricotopus annulator*** **Goetghebuer, 1927** a: head; b: antepronotum; c: scutum and scutellum; d: tip of front tibia; e: tip of middle tibia; f: tip of hind tibia; g: abdominal tergites; h: hypopygium; i: inner lobes of gonocovite.

on V, 22 on VI, 24 on VII, and 20 on VIII, and those on II to VI are arranged roughly into the lateral and the posterior groups, and with a few median setae on IV to VI. Hypopygium in Fig. 18 h. Anal point absent, ninth tergite with about 10 short setae in the median portion. Inner lobe of gonocoxite (Fig. 18 i) composed of three processes, the basal one longest, rounded and clothed with stout and pointed setae, the middle one rectangular and also with strong setae, the distal one small and rounded. Gonostylus simple, not expanded in the middle and without preapical tooth, apical spur prominent.

Remarks. The above stated body coloration, especially the distribution of pale areas on abdominal tergites and legs, is almost coincident with that of *C. annulator* described by Hirvenoja (1973) and Pinder (1987) with the European specimens. The structure of hypopygium, especially that of inner lobes of gonocoxite, is also quite similar to the figures given in these references, but the presence of the third small process found in the present specimens are not drawn with the European specimens, and if it is really absent, the present specimens could be a different new species. The present specimens are also closely related to *C. tokunagai* Hirvenoja, 1993, which was originally recorded from Sakhalin by Tokunaga (1940) with the name of *C. bituberculatus,* in body coloration and in the structure of inner lobes of gonocoxite, and the identity of the latter species with *C. annulator* needs to be studied in future.

19. *Cricotopus* (*Cricotopus*) *bicinctus* (Meigen, 1818)

Nine males were collected at Karakorum (# 27) on August 18, 1996. No. 309:17, 21-23; 311:61-65. This species was originally described from Europe, and also recorded from a number of localities in Japan. It is especially characteristic in body coloration, scutum, scutellum and postnotum almost evenly dark brown, femora entirely brown, tibiae with a long pale ring, tarsi brown, abdominal tergites I and IV entirely white and other tergites almost entirely brown, setae on abdominal tergites II to VI are reduced and arranged roughly into the median and the lateral groups, and inner lobe of gonocoxite is single and foot-shaped.

20. *Cricotopus* (*Cricotopus*) *mongolpequeus* sp. nov. (Figs. 19 a-j)

Three males were collected at Bogd on August 13. 1996 (# 11). Holotype: No. 309:01. Paratypes: No. 309: 02, 03.

Male. BL 3.96, 4.14, 4.08 mm, WL 1.64, 1.74, 1.78 mm, WW/WL 0.32, 0.32, 0.33. Ground color of scutum yellow, stripes dark brown, scutellum brown, postnotum dark brown, leg segments and abdominal tergites almost entirely brown. Head in Fig. 19-a. Eyes pubescent, each with a dorsomedial projection, ER 0.84, 1.18, 1.00. Antenna with 13 flagellar segments, AR 2.03, 2.11, 1.83, AHR 0.55, 0.54, 0.55. Palp short, P/H 0.69, 0.76, 0.69. SO 1+4:2+4, 1+4:2+4, 1+4:1+4. CL all 6. Antepronotum (Fig. 19 b) united in the middle, with 4:5, 4:4, 6:6 lateral setae. Distribution of setae on scutum and scutellum in Fig. 19 c; DM 20, 20, 24, all minute; DL 28:28, 24:24, 24:24, all minute, decumbent and arising on small pits. PA all 4, SC 8, 10, 8, all stout and long.

Wing bare, very finely granular, venation in Fig. 19 d. Costa does not extend beyond tip of R4+5, which is distal to tip of Cul, R/Cu 1.06, 1.04, 1.06. VR 1.14, 1.14, 1.15, RR 0.53, 0.52, 0.54. Cu2 almost straight. Tip of front tibia (Fig. 19 e) with a long apur, 62 μm and 1.6 times as long as the diameter of front tibia at the tip. Tip of middle tibia (Fig. 19 f) with two short spurs. Tip of hind tibia (Fig. 19 g) with a long spur (60 μm), a short spur (12 μm), and a comb composed of 14 free simple spurs 30-63 μm long. fLR 0.53, 0.52, 0.54, mLR 0.44, 0.42, 0.43, hLR 0.52, 0.49, 0.49, all very small. fTR all 0.13, fBR 2.3, 2.5, 2.0, mBR 2.4, 2.7, 2.5, hBR 2.3, 3.0, 2.7. Pulvilli absent.

Distribution of setae on abdominal tergites in Fig. 19 h, those on II to VI are arranged into the median and the lateral groups, the numbers are 42 on I, 38 on II, 36 on III, IV, VI, VII and VIII, and 40 on V in No. 309:03. Hypopygium in Figs. 19 i, j. Anal point absent, ninth tergite with 10 short setae in the median portion. Inner lobe of gonocoxite longer than wide and rounded, bearing short setae. Gonostylus simple, inner margin strongly expanded.

Remarks. This species is a typical member of subgenus *Cricotopus* van der Wulp, 1874, and is especially characterised in that legs and abdominal tergites are almost uniformly brown, gonocoxite with a single thumb-like inner lobe with rounded margin, and inner margin of gonostylus is strongly expanded (Pinder, 1978). Among the species of this subgenus recorded from Europe, this species is somewhat related to *C. pallidipes* Edwards, 1929, in body coloration being almost entirely brown, but differs from it in that inner lobe of gonocoxite is longer, narrower and with numerous short marginal setae, inner margin of gonostylus is more broadly rounded, and AR is larger (1.36 in *C. pallidulus,* after Hirvenoja, 1973, p. 243; Pinder, 1978, Fig. 100C). Among the species recorded from Japan, the present species is similar in body coloration and structure to *C. togaspadix* Sasa et Okazawa, 1992, but in the latter inner lobe of gonocoxite is boot-shaped, abdomi-

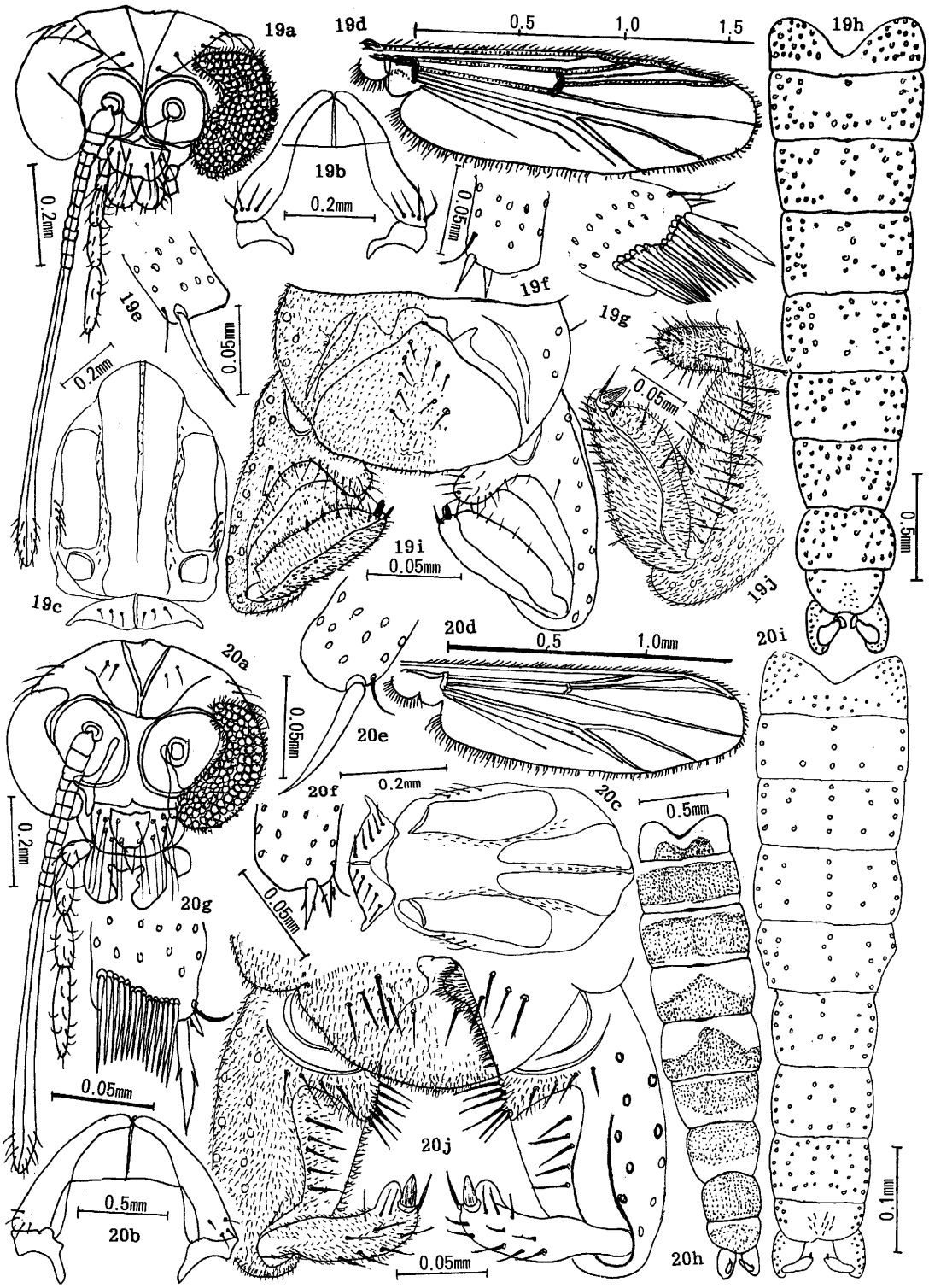

Fig. 19 ***Cricotopus mongolpequeus*** **sp. nov.** a: head; b: antepronotum; c: scutum and scutellum; d: wing; e: tip of front tibia; f: tip of middle tibia; g: tip of hind tibia; h: abdominal tergites; i: hypopygium; j: inner lobe of gonocoxite and gonostylus. **Fig. 20** ***Cricotopus mongolquereus*** **sp. nov.** a: head; b: antepronotum; c: scutum and scutellum; d: wing; e: tip of front tibia; f: tip of middle tibia; g: tip of hind tibia; h: pale and dark areas on abdominal tergites; i: distribution of setae on abdominal tergites; j: hypopygium.

nal tergites III to V with more numerous setae distributed almost evenly, gonostylus is not expanded, and AR is smaller (1.00-1.12). It is also related in body coloration to *C. carbonarius* Kieffer, 1923 recorded by Tokunaga (1936c) from Taiwan, but the latter has large pulvilli and probably belongs to a different subgenus, *Isocladius* Kieffer, 1909.

21. *Cricotopus* (*Cricotopus*) *mongolquereus* sp. nov. (Figs. 20 a-j)

Eleven males were collected at Bogdt (# 10) on August 13, 1996. Holotype: No. 309 : 13. Paratypes: No. 309 : 14, 15, 27, 30-32; 311 : 49-52.

Male. BL 3.12-4.34 (3.51 in average of 6) mm, WL 1.50-2.12 (1.72) mm, WW/WL 0.30-0.33 (0.32). Ground color of scutum yellow, stripes brown, scutellum yellow, postnotum brown. Femora largely pale and with a distal brown ring, tibiae largely pale and with a proximal and distal brown ring, front tarsi brown, middle and hind tarsi I and II yellow, the distal tarsomeres brown. Distribution of pale and dark areas on abdominal tergites in Fig. 20 h; I with a caudal dark area, II and III largely dark and with pale bands along oral and caudal margins, IV and V with an oral pale area and a caudal dark area which is produced orally towards middle, VI and VII largely dark and with a pale band along caudal margin, VIII entirely dark.

Head in Fig. 20 a. Eyes pubescent, with a wedge-shaped dorsomedial extension, ER 0.83-1.07 (0.99). Antenna with 13 flagellar segments, AR 1.37-1.82 (1.53), AHR 0.52-0.56 (0.54). P/H 0.89-1.05 (0.95). SO composed of 0-2 median and 4-8 lateral setae. CL 10-18 (13.3). Antepronotum (Fig. 20 b) united in the middle, with 3 or 4 lateral setae. Distribution of setae on scutum and scutellum in Fig. 20 c. DM 16-28 (19.3), DL 15-32 (22.6), both all minute and arising from very small pits. PA 3-6 (4.3), SC 6-11 (8.8), both well developed. Wing bare, venation in Fig. 20 d. Squama with 10-22 (13.3) fringe hairs. Anal lobe expanding inwards. RR 0.48-0.56 (0.52), VR 1.11-1.17 (1.14), R/Cu 1.04-1.08 (1.06). Tips of tibiae in Figs. 20 e, f, g. fLR 0.52-0.55 (0.54), mLR 0.42-0.47 (0.44), hLR 0.49-0.54 (0.52), fTR 0.13-0.14, fBR 1.4-2.0 (1.7), mBR 2.0-2.7 (2.3), hBR 2.3-3.0 (2.6). Pulvilli absent.

Distribution of setae on abdominal tergites in the holotype in Fig. 20 i, 48 on I, 11 on II, 18 on III and IV, 20 on V, VI and VII, and 35 on VIII, and those on II to VI are arranged into the median and the lateral groups. Hypopygium in Fig. 20 j. Anal point absent, ninth tergite with some 10 setae in the middle portion. Inner lobe of gonocoxite acutely angulate, and bears strong setae on inner margin. Gonostylus simple, with a rounded preapical tooth.

Remarks. This species belongs to the group with one inner lobe on gonocoxite, and it is most closely related to *C. festivellus* (Kieffer, 1906) among the European species of this genus, since inner lobe of gonocoxite is acutely angulate, fore tarsi uniformly dark, all tibiae with conspicuous median pale ring, and setae on abdominal tergites are highly reduced (Hirvenoja, 1973; Pinder, 1978). However, the coloration of abdominal tergites of the latter is quite different from that of the present species, and tergite IV has a pale band along posterior margin in *C. festivellus,* while in the present species, pale band is along the anterior margin of IV and VI, and the dark bands in the posterior part of these tergites are produced anteriorly in the middle. The distribution of setae on abdominal tergites III and IV is also different, in 5 longitudinal lines in the present species, while it is distributed roughly on two transverse lines in *C. festivellus.*

22. *Cricotopus* (*Cricotopus*) *tremulus* (Linnaeus, 1758) (Figs. 21 a-i)

Two males were collected at the side of River Orkhon (# 34, 37) on August 18, 1996, 1,775 m high from sea level. No. 309 : 09, 10. BL 4.20, 4.24 mm, WL 1.57, 1.54 mm, WW/WL 0.31, 0.31. Ground color of scutum dark brown, stripes, scutellum and postnotum black, femora largely dark brown, tibiae with a basal and apical dark ring and a long pale area between them, fore tarsus I brown, entire length of II and basal half of III pale, IV and V brown. Abdominal tergites I and II white, other tergites largely dark. Head in Fig. 21 a. Eyes pubescent, each with a dorsomedial extension, ER 0.74, 0.61. Antenna with 13 flagellar segments, AR 1.57, 1.55, AHR 0.60, 0.54. Palp long, P/H 1.14, 1.23. SO 15: 15, 12 : 12, CL 16, 20. Antepronotum (Fig. 21 b) united in the middle, PN all 2. Distribution of setae on scutum and scutellum in Fig. 21 c. DM both 24, DL all 34, both all minute. PA all 5, SC both 12.

Wing bare, venation in Fig. 21 d. SQ 16 : 18, 20 : 20, RR 0.53, 0.57, VR 1.15, 1.14, R/Cu 1.04, 1.05. Tips of tibiae in Fig. 21 e. f. g. fLR 0.58, 0.59, mLR 0.47, 0.49, hLR 0.51, 0.52, fTR 0.12, fBR 2.3, mBR 2.5, hBR 2.7. Pulvilli absent. Setae on abdominal tergites II to VII are distributed roughly into the lateral, the anterior, and the posterior groups, the numbers being 36 on I, 40 on II and III, 44 on IV and V, and 48 on VI to VIII (Fig. 21 h). Hypopygium in Fig. 21 i. Anal point absent. Inner lobe of gonocoxite single and foot-shaped. Gonostylus with a broad and rounded preapical tooth.

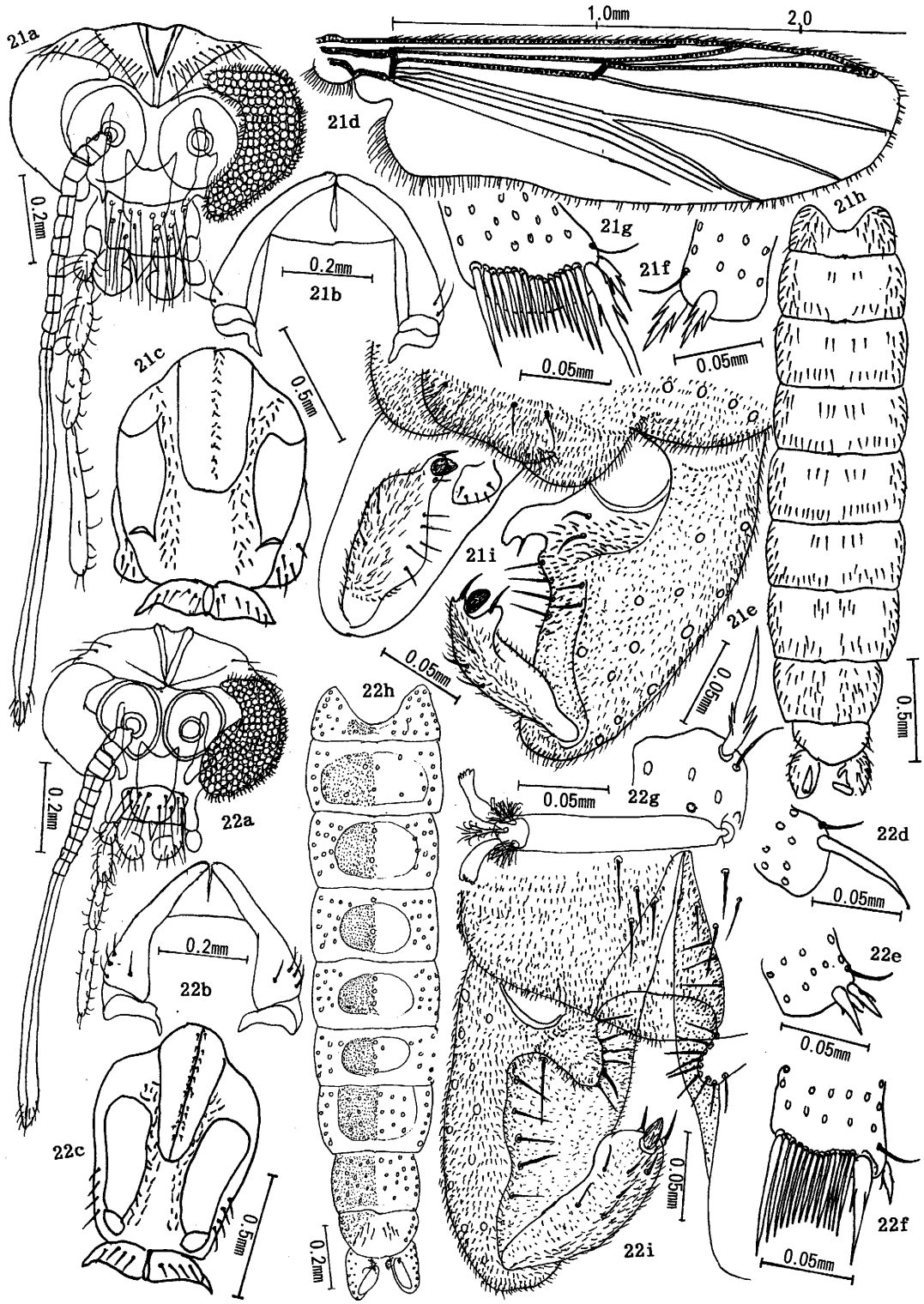

Fig. 21 ***Cricotopus tremulus*** **(Linnaeus, 1758)** a: head; b: antepronotum; c: scutum and scutellum; d: wing; e: tip of front tibia; f: tip of middle tibia; g: tip of hind tibia; h: abdominal tergites; i: hypopygium. **Fig. 22** ***Cricotopus mongolreseus*** **sp. nov.** a: head; b: antepronotum; c: scutum and scutellum; d: tip of front tibia; e: tip of middle tibia; f: tip of hind tibia; g: front tarsus V; h: abdominal tergites; i: hypopygium.

Remarks. The above body coloration, measurement data and structure of the present specimen are almost in accordance with those of *C. tremulus* (Linnaeus) described by Hirvenoja (1973) and Pinder (1978) from Europe, and also by Tokunaga (1940) from Sakhalin. This species is especially characteristic in the coloration of abdominal tergites, I and II pale and other tergites dark, and of the fore tarsi, entire length of II and proximal half of III are white and other portions dark. The shape of inner lobe of gonocoxite being leg-form is another distinguishing character of this species.

23. *Cricotopus* (*Cricotopus*) *triannulatus* (Macquart, 1826)

Twelve males were collected at Karakorum on August 18, 1996 (#26). No. 309 : 04-07; 311 : 53-60. The body coloration, measurement data and external structure of the above specimens are almost coincident with those of *C. triannulatus* described by various authors from Europe, and also by Tokunaga (1936c) and by Sasa and Kikuchi (1995, p. 55) from a number of localities in Japan. Especially characteristic is the body coloration, femur is largely brown, tibiae largely pale and with basal and apical dark rings, abdominal segment II with a broad pale band in oral half, IV and V are largely pale, inner lobe of gonocoxite is composed of an anterior and a posterior arm, the former is sharply angulate, and gonostylus is strongly expanded in the middle.

24. *Cricotopus* (*Isocladius*) *mongolreseus* sp. nov. (Figs. 22 a-i)

A male was collected at Bogd (#10) on August 13, 1996. Holotype: No. 309 : 11.

Male. BL 3.48, WL 1.56, WW/WL 0.34. Ground color of scutum yellow, stripes dark brown, scutellum yellow, postnotum dark brown, proximal half of femora yellow, distal half brown, tibiae largely yellow and with narrow brown rings at both ends, front tarsi uniformly brown, entire length of middle and hind tarsi I, II and proximal half of tarsus III yellow, the rest tarsal segments brown. Abdominal tergites II to VII each with an oval brown area in the middle, VIII largely brown (Fig. 22 h).

Head in Fig. 22 a. Eyes pubescent, ER 0.91, AR 1.60, AHR 0.56. P/H 0.84. SO 0+3, 0+3, CL 12. Antepronotum (Fig. 22 b) united in the middle, with only one tiny lateral seta. Distribution of setae on scutum and scutellum in Fig. 22 c. DM 24, DL 24 : 24, both all minute and arising from small pits. PA 4 : 4, SC 8. SQ 8 : 10. RR 0.57, VR 1.16, R/Cu 1.06. fLR 0.59 mLR 0.45, hLR 0.53, fTR 0.15, fBR 2.7, mBR 2.5, hBR 2.7. Tips of tibiae in Figs. 22 d, e, f. Tip of tarsomeres V with an empodium, a pair of claws and a brush-like pulvilli (Fig. 22 g, front tarsus V).

Distribution of setae on abdominal tergites in Fig. 22 h, 36 on I, 22 on II,26 on III to VII, and 32 on VIII, and those on III to VI are arranged into 2 to 4 median and the rest in lateral groups. Hypopygium in Fig. 22 i. Anal point absent, ninth tergite with 10 short setae in the median portion. Inner lobe of gonocoxite single, about as long as wide and rounded, with strong setae on inner margin. Gonostylus strongly expanded near apex.

Remarks. This species is regarded as belonging to the subgenus *Isocladius,* since legs with well developed pulvilli. According to Pinder (1978), European species of this subgenus is divided into two groups according to the shape of basal portion of inner margin of gonocoxite, either rounded or produced into a hump, but in the present species it is straight. The setae on abdominal tergites II to V are arranged roughly into the lateral and the posterior rows, and does not fit to that of any European species. The peculiar coloration of abdominal tergites, II to VI with a large oval dark mark, also differs from the previously known species of this subgenus.

25. *Cricotopus* (*Isocladius*) *sylvestris* (Fabricius, 1794)

A male was collected at Karakorum (#27) on August 18, 1996. No. 309 : 08. BL 4.66 mm, WL 2.26 mm, WW/WL 0.30. Ground color of scutum pale, stripes dark brown, median stripes reaching to only about middle of scutum, scutellum brown, postnotum dark brown, femora basally brown and distally dark brown, tibiae largely yellow and with a basal and a distal brown ring, front tarsomeres entirely brown, middle tarsomere I largely yellow with a basal and an apical dark ring, hind tarsomere I largely yellow and with a basal brown ring, the distal tarsomeres brown. Abdominal tergite I entirely pale, IV largely pale and with a small brown spot in the middle, VII largely pale and with a brown band along anterior margin, II, III, V and VI largely brown and with pale bands along anterior and posterior margines, VIII and IX entirely brown.

Eyes pubescent, each with a wedge-shaped dorsomedial extension, ER 1.00. Antenna with 13 flagellar segments, AR 1.74, AHR 0.54. P/H 1.03, SO 1+4 : 1+4, CL 10, PN 5 : 5. DM 18, DL 18 : 20, both all minute. PA 5: 5, SC 10, all well developed. SQ 24 : 24, anal lobe expanding inwards. RR 0.53, VR 1.17, R/Cu 1.07. fLR 0.58, mLR 0.45, hLR 0.51, fTR 0.16, fBR 2.0, mBR 0.21,

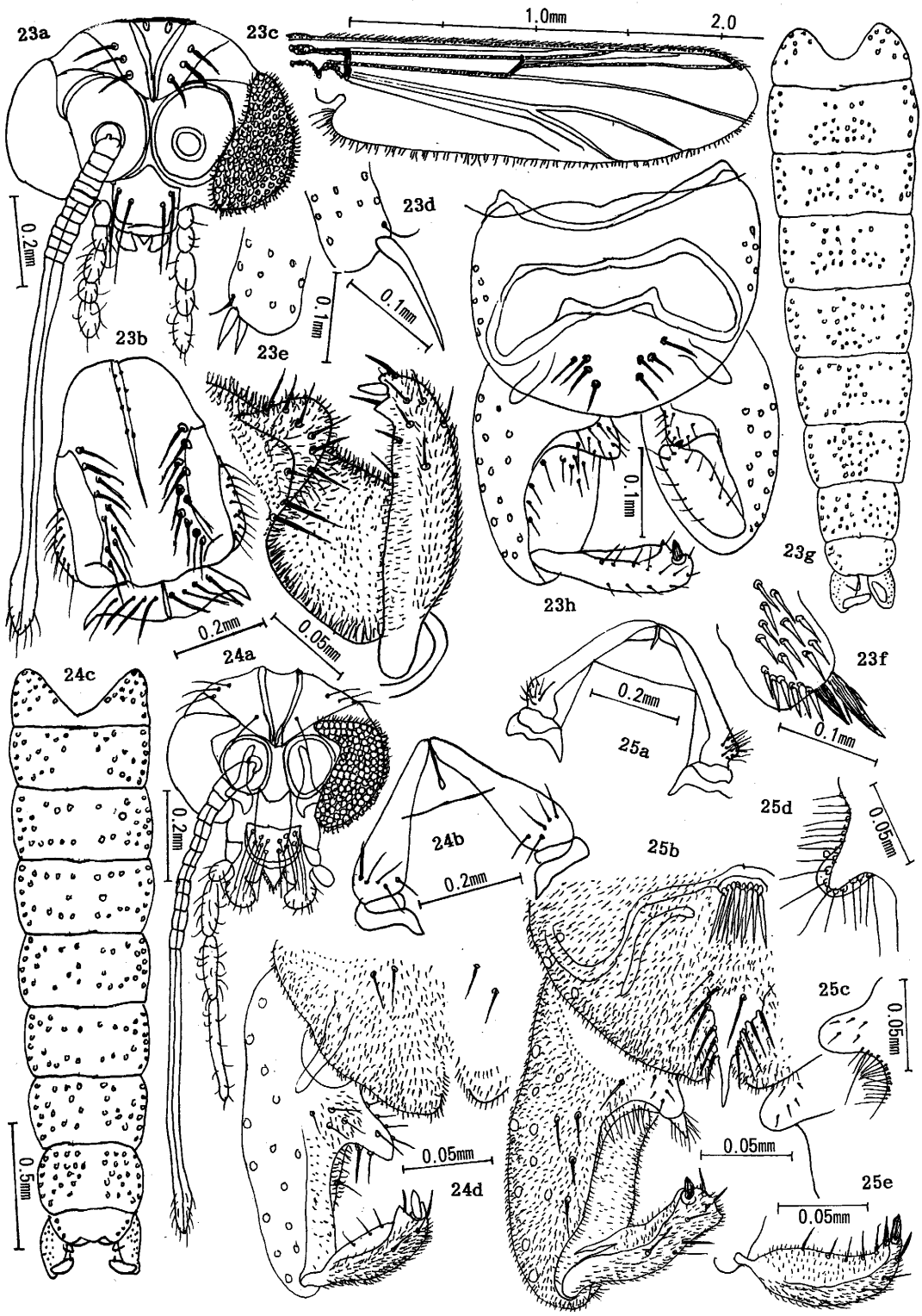

Fig. 23 ***Paratrichocladius mongolseteus*** **sp. nov.** a: head; b: scutum and scutellum; c: wing; d: tip of front tibia; e: tip of middle tibia; f: tip of hind tibia; g: abdominal tergites; h: hypopygium; i: inner lobe of gonocoxite, and gonostylus. **Fig. 24** ***Paratrichocladius rufiventris*** **(Meigen, 1830)** a: head; b: antepronotum; c: abdominal tegites; d: hypopygium. **Fig. 25** ***Orthocladius uraanbaatur*** **sp. nov.** a: antepronotum; b: hypopygium; c: inner lobe of gonocoxite, dorsal view; d: inner lobe of gonocoxite, ventral view; e: gonostylus.

hBR 0.22. Pulvilli large, brush-like. Setae on abdominal tergites II to VI are arranged roughly into the median and the lateral groups. Anal point absent, inner lobe of gonocoxite single, rounded and bearing strong setae, inner margin of gonostylus expanded in the middle.

Remarks. The above stated body coloration, measurement data and the structures indicate that the present specimen belongs to the pale form of *C. sylvestris,* a cosmopolitan species recorded from many lakes in the world.

26. *Paratrichocladius mongolseteus* sp. nov.

(Figs. 23 a-i)

A male was collected at Bogd (# 18), about 1,500 m high from sea level, on August 13, 1996. No. 309 : 45

Male. BL 4.42 mm, WL 2.24 mm, WW/WL 0.30. Scutum, scutellum and postnotum entirely black, only the bases of DL setae are pale. Leg segments and abodominal tergites almost evenly dark brown. Head in Fig. 23 a. Eyes pubescent, reniform and without dorsomedial projection, ER 1.43. Antenna with 13 flagellar segments, AR 2.45, AHR 0.66. Palp unusually short, P/H only 0.51. SO 3 : 3, all arising in the median portion. CL only 4. Antepronotum lost. Distribution of setae on scutum and scutellum in Fig. 23 b. DM 8, all minute. DL 10 : 10, all well developed and arising from large pale pits. PA 10 : 10 (all very thin). SC 8.

Wing bare, very finely granular, venation in Fig. 23 c. Squama lost. Anal lobe strongly produced inwards. Costa does not extend beyond tip of R4+5. R2+3 separated, RR 0.63. VR 1.09, R/Cu 1.13. Cu2 almost straight. Tip of front tibia (Fig. 23 d) with a long and curved spur, 88 μm long and 1.5 times as long as the diameter of front tibia at the tip. Tip of middle tibia (Fig. 23 e) with two short spurs, both 20 μm long. Tip of hind tibia (Fig. 24 f) with a long (38 μm) and a short (17 μm) spur, and with a terminal comb of only 6 spines, and other 12 spines are distributed in the distal portion. Tarsi I and II of middle and hind legs without preapical spur. fLR 0.69, mLR 0.46, hLR 0.56, fTR 0.18, fBR 2.0, mBR 2.0, hBR 2.0. Pulvilli absent.

Setae on abdominal tergites (Fig. 24 g) are arranged roughly into the lateral and the median groups, 16 on I, 36 on II, 40 on III to VII, and 36 on VIII. Hypopygium in Fig. 23 h. Anal point and virga absent. Ninth tergite bearing 8 short setae in the middle portion. Inner lobe of gonocoxite prominent, nearly rectangular but with rounded margin, with strong marginal setae. Gonostylus (Fig. 23 i) simple, with small rectangular preapical tooth.

Remarks. This specimen is considered as belonging to the genus *Paratrichocladius* Santos Abreu, 1918, since eyes are pubescent, dorsolateral setae of scutum is well developed and arising from large pale pits, wing venation is the *Cricotopus* type and costa does not extend beyond tip of R4+5, which is distal to tip of Cu1, Cu2 is nearly straight, and anal point is absent. Species of this genus have fringe hairs on squama, but unfortunately both squamae are lost. This species is however quite unusual as a member of this genus in that maxillary palp is very short, P/H only 0.51, and terminal comb of hind tibia is composed of only 6 spines.

27. *Paratrichocladius rufiventris* (Meigen, 1830)

(Figs. 24 a-d)

Nine males were collected at Karakorum (# 30) on August 18, 1996. No. 309 : 34-49; 311 : 72-74. BL 2.78-3.32 (3.06 in average of 6) mm, WL 1.51-1.85 (1.72 in average of 9) mm, WW/WL 0.31-0.33 (0.32). Ground color of scutum brown, stripes, scutellum and postnotum dark brown, legs and abdomen uniformly brown. Head in Fig. 24 a. Eyes pubescent, each with a conspicuous dorsomedial extension, ER 0.50-0.67 (0.60). Antenna with 13 flagellar segments, AR 1.14-1.42 (1.28), AHR 0.52-0.56 (0.54). P/H 1.04-1.26 (1.18), SO composed of 1 or 2 inner and 4 or 5 lateral groups. CL 12-16 (14.5). Antepronotum (Fig. 24 b) united in the middle, with 4 or 5 (4.5) lateral setae. DM 8-10 (9.0), DL 10-14 (12.6), all well developed. PA 3 or 4 (3.3), SC 10-14 (12.0), SQ 8-12 (9.5), RR 0.48-0.54 (0.52), VR 1.09-1.13 (1.11), 1.03-1.05 (1.04), fLR 0.58-0.62 (0.60), mLR 0.49-0.51 (0.50), hLR 0.57-0.59 (0.58), fTR 0.13-0.14, fBR 2.2-2.6 (2.4), mBR 2.6-3.0 (2.8), hBR 2.6-3.2 (2.9). Pulvilli small, brush-like.

Distribution of setae on abdominal tergites as in Fig. 24 c, the numbers are 28 on I, 32 on II to VII, and 40 on VIII, and those on II to VII are arranged roughly into 7-10 lateral groups and the anterior and posterior rows. Hypopygium in Fig. 24 d. Anal point absent. Inner lobe of gonocoxite acutely angulate. Gonostylus with a large, rectangular preapical tooth.

Remarks. The above measurement data and structure of the present specimens are almost coincident with those of *P. rufiventris* (Meigen, 1830), which are commonly recorded from Europe and Japan. Its morphology was described in details by Sasa (1981) in comparison with the related species, *P. tamaater* Sasa, 1981, and is especially characterised by that abdominal tergites II to VI with 7 or more lateral setae (only 4 in *P. tamaater*). The present specimens also differs from the above species, *P. mongolseteus,* in that palp is long, eyes with a long dorsomedial extension with smaller ER

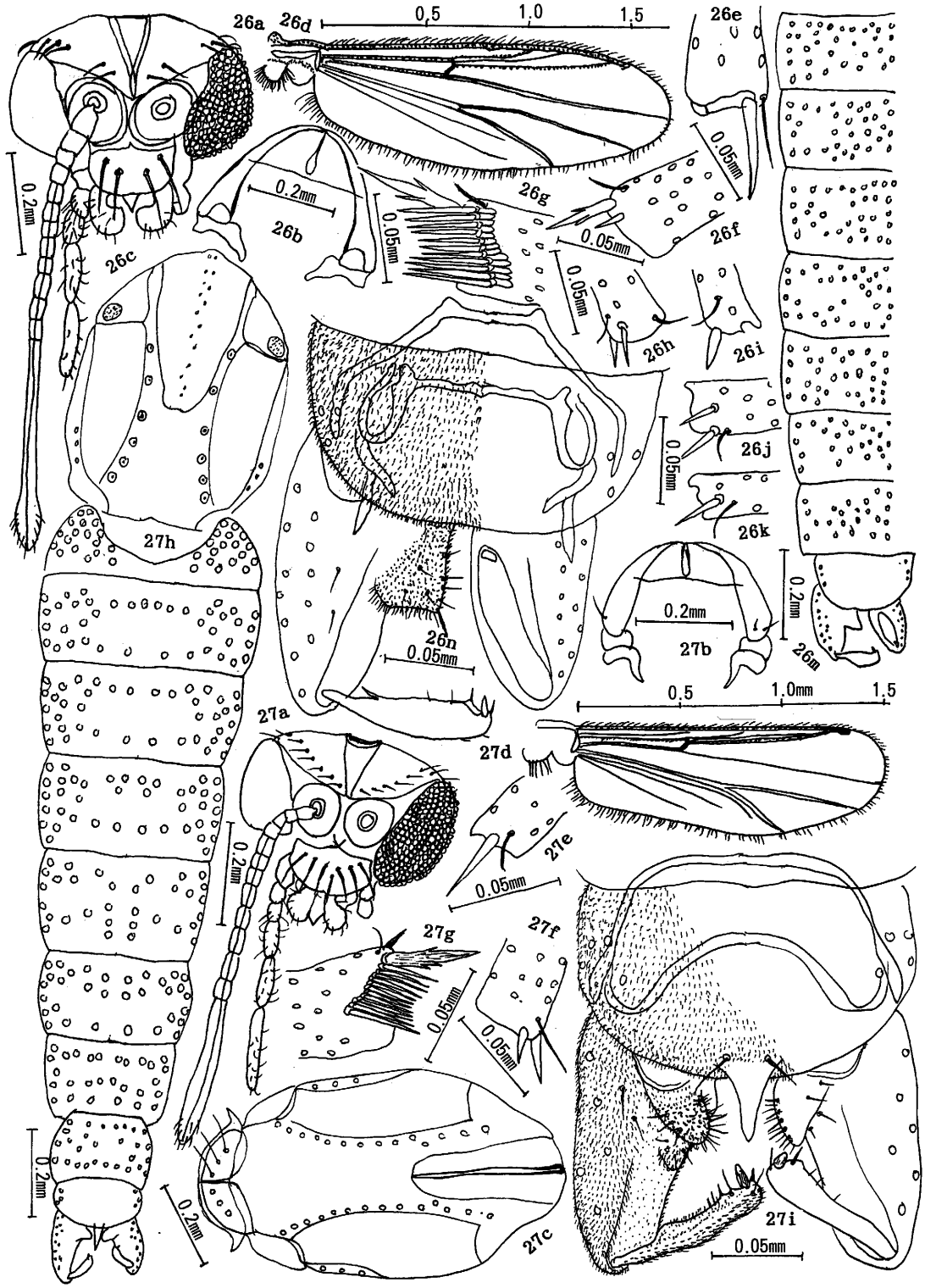

Fig. 26 ***Eukiefferiella mongolteuus*** **sp. nov.** a: head; b: antepronotum; c: scutum; d: wing; e: tip of front tibia; f: tip of middle tibia; g: tip of hind tibia; h: tip of middle tarsus I; i: tip of middle tarsus II; j: tip of hind tarsus I; k: tip of hind tarsus II; m: abdominal tergites; n: hypopygium. **Fig. 27** ***Eukiefferiella mongoluveus*** **sp. nov.** a: head; b: antepronotum; c: scutum and scutellum; d: wing; e: tip of front tibia; f : tip of middle tibia; g: tip of hind tibia; h: abdominal tergites; i: hypopygium.

values, and hind tibia with 10 or more terminal comb spines.

28. *Orthocladius* (*Orthocladius*) *ulaanbaatus* sp. nov. (Figs. 25 a-e)

A total of 33 males and 17 females were collected at Ulaanbaatur on August 1, 1995. Holotype: male, No. 306 : 05. Paratypes: males, No. 306 : 06-23, 84-88; 311 : 01-09; females: 306 : 24-29, 90-100.

Male. BL 3.26-3.58 (3.39 in average of 8) mm, 1.66-2.16 (1.85 in 8) mm, WW/WL 0.31-0.34 (0.32). Scutum largely brown, with the exception of humeral areas being yellow, scutellum brown, postnotum dark brown, legs almost uniformly brown; abdominal tergites II to V brown for median and lateral areas and brownish yellow for both sides of median dark area, VI to VIII largely brown and with a yellow band along caudal margin. Eyes bare, each with a dorsomedial projection, ER 0.97-1.32 (relatively high). Antenna with 13 flagellar segments, AR 1.34-1.48 (1.41, relatively small). Palp short, P/H 0.92-0.95 (0.94). SO 8-16, most frequently 12, mean 12.3. CL 8-14 (12.1). Antepronotum (Fig. 25 a) very narrowly united in the middle, with 9-12 (most frequently 10, mean 10.8, very many) lateral setae. DM 12-20 (14.3), all minute. DL 6-10 (most frequently 8, mean 8.4), PA 4-6 (4.5), SC 8-12 (10.0).

Wing bare, brownish and very finely granular, anal lobe strongly produced inwards. Costa does not extend beyond tip of R4+5. RR 0.33-0.42 (0.38), VR 1.06-1.12 (1.10), R/Cu 1.03-1.08 (1.06). Cu2 almost straight, slightly curved near apex. fLR 0.61-0.64 (0.62), mLR 0.45-0.47 (0.46, very small), hLR 0.54-0.57 (0.56), fTR 0.15-0.17 (0.16), fBR 1.9-2.7 (2.4), mBR 2.6-5.2 (3.4), hBR 2.6-5.4 (4.6). Tarsi I and II of middle leg, and tarsus I of hind leg each with 2 subterminal spurs, other tarsomeres, including II of hind leg, without subterminal spurs. Pulvilli absent.

Hypopygium in Fig. 25 b. Anal point widest at base, with lateral setae, and distal half narrow and with sharply pointed apex. Virga 30 μm long and 20 μm wide, composed of some 20 strong spines. Inner lobes of gonocoxite Fig. 25 c, dorsal, 25 d, ventral) are composed of almost completely overlapping two layers, the dorsal one broader and rounded, the ventral one narrower and angulate. Gonostylus (also in Fig. 25 e) simple, without preapical tooth, nearly straight and with rectangularly truncate apex, or widest at about middle and tapering towards apex (the shape of such differences are probably due to the angle of observations).

Remarks. The above structure and measurement data indicate that these specimens belong to the *gla-bripennis* group of subgenus *Orthocladius* which are at present rather difficult to be classified into separate species with adult males (Sasa and Kikuchi, 1995, p. 170). The structures and measurement data of the above specimens collected in Mongol are closely related to those obtained with specimens of this group collected in the summer season in Japan, but they are considered as a different new species, because the body size, the values of AR, mLR and the numbers of most scutal setae are smaller, and the numbers of lateral setae of antepronotum are 9-12 in the present specimens and much larger than 3 to 6 obtained with the Japanese specimens examined by us (Sasa and Kikuchi, 1995, p. 170).

29. *Eukiefferiella mongolteuus* sp. nov. (Figs. 26 a-n)

A male was collected at Orkhon Gol on August 18, 1996 (# 45) at an altitude of 1,775 m. Holotype: No. 306: 02.

Male. WL 1.77 mm, WW/WL 0.34 (very wide). Scutellum and postnotum are lost from the mounted specimen. Ground color of scutum yellow, stripes brown, legs brownish yellow, abdominal tergites brown. Head in Fig. 26 a. Eyes bare, reniform, inner margin concave but without dorsomedial extension, ER 1.37. Antennal flagellum 13 segmented, AR 0.86, AHR 0.45, apical portion strongly expanded, without apical seta. Palp short, P/H 0.88. SO 2+4 : 2+4, CL 4. Antepronotum (Fig. 26 b) very narrowly united in the middle, without setae. Distribution of setae on scutum in Fig. 26 c. DM 16, all minute. DL 6 : 6, all arising from large pale pits. PA 3:3.

Wing bare, membrane brownish and slightly granular, venation in Fig. 26 d. Squama with 20 : 20 fringe hairs, anal lobe obtuse. Costa extending slightly beyond tip of R4+5, which is proximal to tip of Cu1, R/Cu 0.93. R2+3 separated, RR 0.34. VR 1.16. Cu2 nearly straight. Tip of front tibia (Fig. 26 e) with a long spur, 56 μm long and 1.6 times as long as the diameter of front tibia at the tip. Tip of middle tibia (Fig. 26 f) with two spurs, one longer (30 μm) and the other shorter (17 μm). Tip of hind tibia (Fig. 26 g) with a long spur (54 μm), a short spur (12 μm), and a comb composed of 12 simple spurs. Tips of middle and hind tarsomere I (Figs. 26 h, j) with two simple subterminal spurs, tips of middle and hind tarsomere II (Figs 26 i, k) with one simple subterminal spur. fLR 0.66, mLR 0.52, hLR 0.58, fTR 0.17, fBR 2.3, mBR 3.2, hBR 4.4. Pulvilli absent.

The numbers of setae on abdominal tergites are relatively large, 64 on II to VI, 48 on VII, and 44 on VIII, as in Fig. 26 m (left half). Hypopygium in Fig. 26 n.

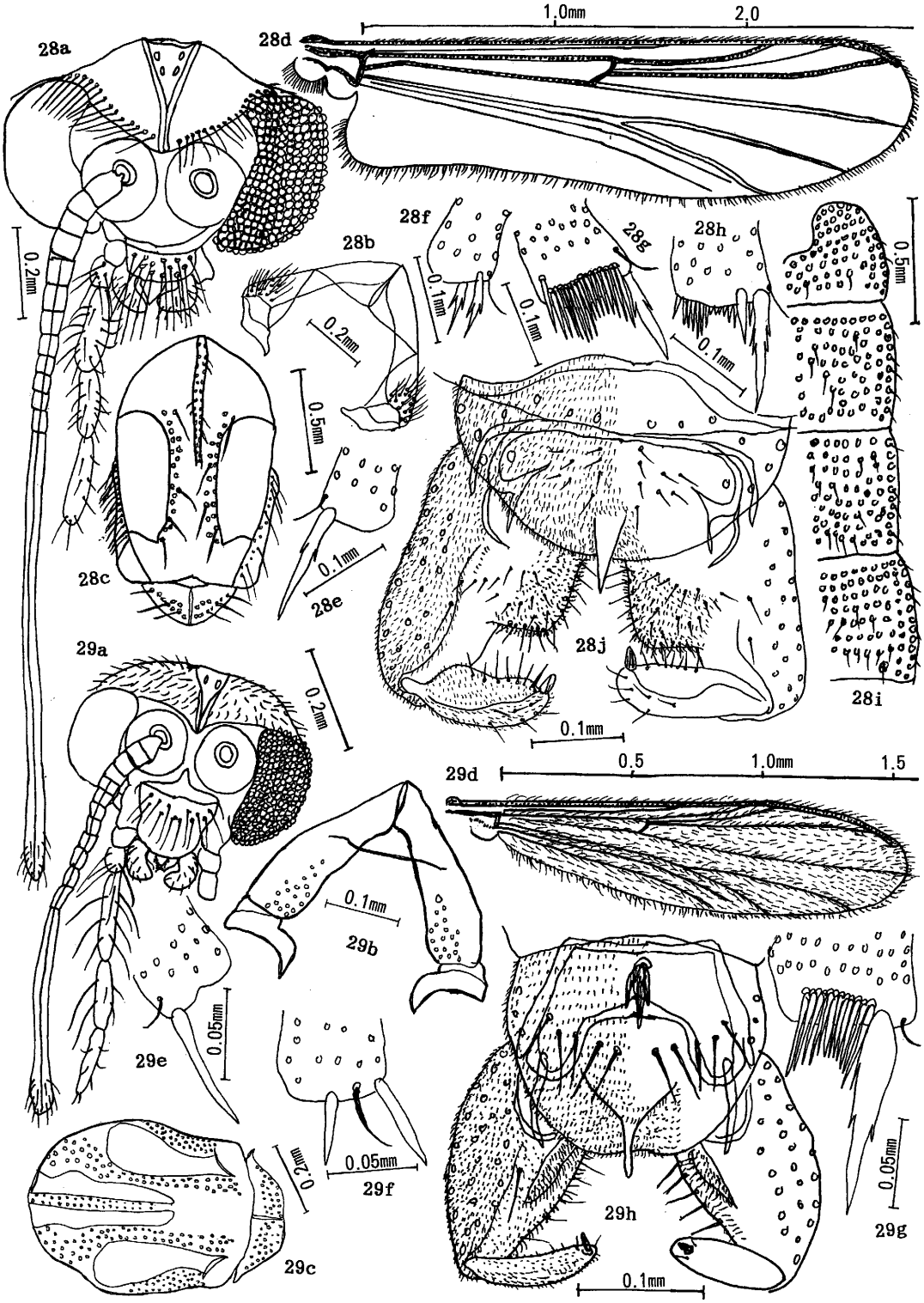

Fig. 28 ***Chaetocladius mongolveweus* sp. nov.** a: head; b: antepronotum; c: scutum and scutellum; d: wing; e: tip of front tibia; f: tip of middle tibia; g, h: tip of hind tibia; i: abdominal tergites I to IV, left half; j: hypopygium.
Fig. 29 ***Metriocnemus oiraquintus* Sasa, 1991** a: head; b: antepronotum; c: scutum and scutellum; d: wing; e: tip of front tibia, f: tip of middle tibia; g: tip of hind tibia; h: hypopygium.

Anal point absent, ninth tergite without long setae in the middle portion. Inner lobe of gonocoxite large and rectangular. Gonostylus simple, with a small preapical tooth.

Remarks. This species is considered as belonging to the genus *Eukiefferiella* Thienemann 1926, in wider sense, and seems to belong to the *chuzeoctava* group of this genus, since eyes are bare, squama fringed, R2+3 is separated from both R1 and R4+5, and anal point is absent, and is very closely related to *E. chuzeoctava* Sasa, 1984, in measurement data and structure (Sasa, 1984, p. 73). However, this specimen is considered as belonging to a different new species, since body coloration is much paler (almost entirely black in *E. chuzeoctava*), eyes with concave inner margin and ER is smaller (inner margin convex, ER 1.41-1.54 in the latter), palpi are shorter and tip of R4+5 is located more proximally (P/H 1.02, R/Cu 0.99, in the holotype of *E. chuzeoctava*). The present specimen has only one terminal spur on tarsomere II of middle and hind legs, which could be another differentiating character from *E. chuzeoctava*, in which tarsomeres I and II of middle and hind legs all have two subterminal spurs.

30. *Eukiefferiella mongoluveus* sp. nov.

(Figs. 27 a-i)

A male was collected at Ulanbaatur on August 1, 1995. Holotype: No. 306 : 03.

Male. BL 2.65 mm, WL 1.52 mm, WW/WL 0.33. Ground color of scutum yellow, stripes and postnotum dark brown, scutellum brownish yellow, legs and abdomen brownish yellow. Head in Fig. 27 a. Eyes bare, reniform, without dorsomedial extension, ER 1.25. Palp with 13 flagellar segments, AR 0.72, AHR 0.43. SO 8 : 8. CL 7. Antepronotum (Fig. 27 b) slightly separated in the middle, with 1 : 2 lateral setae. Distribution of setae on scutum and scutellum in Fig. 27 c. DM 0, DL 13 : 14, PA 3 : 3, SC 6, all well developed.

Wing bare, brownish, venation in Fig. 27 d. SQ with 16 fringe hairs. Anal lobe obtuse. Costa extending slightly beyond tip of R4+5, which is much proximal to tip of Cu1, R/Cu 0.94. R4+5 in contact with R4+5. FCu much distal to R-M, VR 1.47. Tip of front tibia (Fig. 27 e) with a long spur, 43 μm long and 1.6 times as long as the diameter of front tibia at the tip. Tip of middle tibia (Fig. 27 f) with two spurs, 20 and 24 μm long. Tip of hind tibia (Fig. 27 g) with a long (53 μm) and a short (22 μm) spur, and a comb composed of 10 free spines. All tarsal segments without terminal spur. fLR 0.65, mLR 0.46, hLR 0.54, fTR 0.13. Tarsi with long beards, fBR 3.9, mBR 3.2, hBR 6.8. Pulvilli vestigial.

Distribution of setae on abdominal tergites in Fig. 27 h, 36 on I, 40 on II, III and IV, 32 on V and VI, 28 on VII and VIII, and those on II to VII are arranged into the anterior row, the posterior row, and the latarel groups. Hypopygium in Fig. 27 i. Anal point long, widest at base and apically pointed, with a pair of setae on base, otherwise ninth tergite without setae in the middle portion. Virga is absent. Inner lobe of gonocoxite single, longer than wide and rounded. Gonostylus simple, narrow and with a small preapical tooth.

Remarks. This species belongs to the *tamaflava* group of genus *Eukiefferiella*, since R2+3 is in contact with R4+5, tip of R4+5 is proximal to tip of Cu1 and R/Cu <1.0, squama with fringe hairs, and anal point is present (Sasa and Kikuchi, 1995, p. 157). It is most closely related to *E. tamaflava* Sasa, 1981 among the known species of this group, and the shape of eyes, anal point and gonostylus as well as body coloration are quite similar, but *E. tamaflava* differs from the present species in that AR is 0.37 and smaller, the numbers of setae on abdominal tergites are much fewer (only 13-16 on tergites II to VI), virga is present, ninth tergite has a pair of rounded lobes flanking anal point, and inner lobe of gonocoxite is broader (Sasa, 1981). This species differs from the preceding one, *E. mongolseteus*, in that anal point is present, inner lobe of gonocoxite is rounded, antepronotum with a lateral seta, and the setae on abdominal tergites are fewer.

31. *Chaetocladius mongolveweus* sp. nov.

(Figs. 28 a-j)

Seventeen males were collected on Mount Bogdrhan (#6), on an elevation of 2,400 m, on August 5, 1995. Holotype: 306 : 42. Paratypes: 306:43-51, 310:10-16.

Male. BL 4.72-5.28 (5.05 in average of 8) mm, WL 2.88-3.08 (2.97)mm, WW/WL 0.26-0.28 (0.27). Ground color of scutum brownish yellow, stripes dark brown, scutellum brownish yellow, postnotum dark brown, legs and abdominal tergites brownish yellow. Head in Fig.28 a. Eyes bare, each with a wedge-shaped dorsomedial projection, ER 0.44-0.90 (0.70). Antenna with 13 flagellar segments, AR 1.92-2.34 (2.08), AHR 0.58-0.65 (0.62). Palp relatively short, P/H 0.86-0.96 (0.92). SO 12-22 (16.7), CL 6-12 (9.6). Antepronotum (Fig. 28 b) united in the middle, with 8-16 (11.9, very many) lateral setae. Distribution of setae on scutum and scutellum in Fig. 28 c. DM 22-34 (27.0), DL 18-26 (22.7), PA 11-18 (12.8), SC 14-22 (18.3), all very many.

Wing membrane bare but conspicuously granular, venation in Fig. 28 d. Squama with 12-24 (17.6) fringe hairs. R2+3 separated, ending closer to tip of R4+5

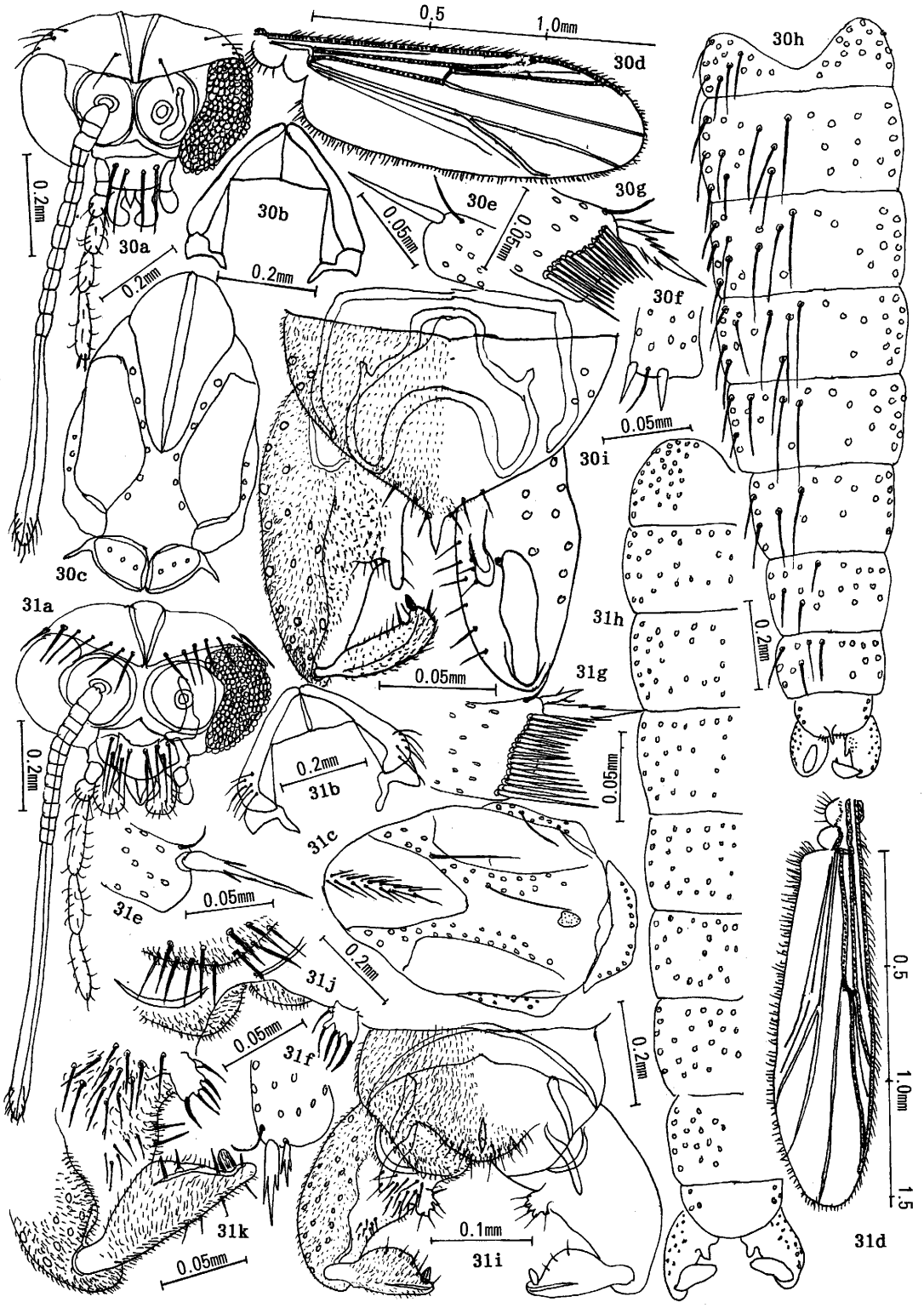

Fig. 30 ***Mongolcladius mongolwexeus*** **sp. nov.** a: head; b: antepronotum; c: scutum and scutellum; d: wing; e: tip of front tibia; f: tip of middle tibia; g: tip of hind tibia; h: abdominal tergites; i: hypopygium. **Fig. 31** ***Mongolyusurika mongolxeyeus*** **sp. nov.** a: head; b: antepronotum; c: scutum and scutellum; d: wing; e: tip of front tibia; f: tip of middle tibia; g: tip of hind tibia; h: abdominal tergites, left half; i: hypopygium; j: caudal portion of ninth tergite; k: inner lobe of gonocoxite, and gonostylus.

than to tip of R1, RR 0.60-0.66 (0.63). VR 1.06-1.12 (1.09), R/Cu 1.06-1.10 (1.08). Costa extending only slightly beyond tip of R4+5. Cu2 nearly straight. Tip of front tibia (Fig. 28 e) with a long spur, 115 μm long and 1.7 times as long as the diameter of front tibia at the tip. Tip of middle tibia (Fig. 28 f) with two spurs, 57 and 50 μm long. Tip of hind tibia (Figs. 28 g, h) with a long (113 μm) and a short (40 μm) spur, and a comb composed of 16 free spines 33-68 μm long. Tips of middle and hind tarsi I and II each with two simple subterminal spurs. Pulvilli absent. fLR 0.63-0.69 (0.66), mLR 0.41-0.45 (0.43), hLR 0.49-0.53 (0.51), fTR 0.12-0.13, fBR 2.8-4.5 (3.5), mBR 3.0-3.7 (3.5), hBR 3.3-4.1 (3.7).

Setae on abdominal tergites (Fig. 28 i, I to IV, right half) are very many but short and weak, and almost evenly distributed, 128 on I, 132 on II, III, and IV, 140 on V and VI, 134 on VII, and 100 on VIII, in the holotype. Hypopygium in Fig. 28 j. Anal point long, widest at base and tapering towards pointed apex, but very faint, without microtricia and without lateral setae. Ninth tergite with some 16 short setae in the middle portion. Virga absent. Inner lobe of gonocoxite single, very broad, obtuse and with rounded corner. Gonostylus simple, widest at about middle, and without preapical tooth.

Remarks. This species is considered as belonging to the genus *Chaetocladius* Kieffer, 1911, since wing is bare and granular, squama fringed, costa not extending much beyond tip of R4+5, pulvilli absent, and anal point is well developed but without lateral setae (Pinder, 1978; Sasa and Kikuchi, 1995). This species is most closely related among the European species of this genus to *C. melaleucus* (Meigen), but in the latter AR is about 1.5 (smaller) and body is entirely black, anal point is much smaller, inner lobe of gonocoxite is narrower, rectangularly angulate and has a hook-like process, and gonostylus is not expanded basally according to Edwards (1929) and Pinder (1978, Fig. 123D). Five species have been recorded from Japan as members of this genus by Sasa and Kikuchi (1995), among which the present one is somewhat related to *C. oyabevenustus* Sasa, Kawai et Ueno, 1988, in that inner lobe of gonocoxite is simple, and anal point is long, and AR is about 2.2, but in the latter pulvilli and virga are present, anal point is apically rounded, and inner lobe of gonocoxite is higher and narrower.

32. *Limnophyes minimus* (Meigen, 1818)

Four males were collected at Bogdrhan on August 5, 1995, 2,400 m above sea level. No. 306 : 53-56. BL 1.92-2.32 (2.13 in avarage of 4) mm, WL 0.98-1.26 (1.09) mm, WW/WL 0.30-0.33 (0.31). ER 1.35-1.65 (1.50). Antennal flagellum 11 segmented in 1, 12 in 2, and 13 in 1, AR 0.60-0.68 (mean 0.64), AHR 0.35-0.51 (0.46). Palp short, P/H 0.78-0.88 (0.83). SO 3-5 (4.0), CL 6-10 (8.5). DM 0 in 3, 4 in 1, DL 9-16 (12.8), PA 5 or 6 (5.8), SC all 4.

Wing bare, granular, SQ 1-3 (2.0), RR 0.31-0.36 (0.34), VR 1.28-1.33 (1.31), R/Cu 1.04-1.09 (1.06). fLR 0.47-0.52 (0.49), mLR 0.43-0.46 (0.45), hLR 0.52-0.58 (0.55), fTR 0.13-0.14, fBR 2.3-2.7 (2.5), mBR 2.5-3.1 (2.9), hBR 3.1-4.3 (3.6).

Remarks. This species is identified as *L. minimus*, since the above measurement data and the structures are within the variation ranges of this species recorded from Europe, and also described in detailes by Sasa and Kikuchi (1986) with the Japanese specimens.

33. *Metriocnemus oiraquintus* Sasa, 1991

(Figs. 29 a-h)

A male was collected at Mt. Bogdrhan on August 5, 1995 (# 7), at about 2,400 m high from sea level. No. 306: 81.

Male. BL 3.06 mm, WL 1.63 mm, WW/WL 0.26 (very narrow). Body almost uniformly dark brown. Head in Fig. 29 a. Eyes bare, reniform and without dorsomedial extension, ER 0.90. Antenna with 13 flagellar segments, AR 1.06, AHR 0.51. Palp well developed, long, P/H 1.17, and setae on segments I to III are unusually long. SO 40 : 40 (very many), CL 12. Antepronotum (Fig. 29 b) united in the middle, with 18 : 18 basolateral setae (very many). Distribution of setae on scutum and scutellum in Fig. 29 c; DM 32, DL 54 : 56. PA 32: 34, SC 46, all very many and well developed.

Wing very narrow, anal lobe nearly flat, membrane thickly clothed with macrotrichia on entire surface, venation in Fig. 29 d. Both squamae are lost from the mounted specimen. Costa slightly extending beyond tip of R4+5, which is slightly distal to tip of Cu1, R/Cu 1.03. R2+3 separated, RR 0.27. VR 1.29. Cu2 nearly straight. Tip of fore tibia (Fig. 29 e) with a long spur, 76 μm long and 1.5 times as long as the diameter of fore tibia at the tip. Tip of middle tibia (Fig. 29 f) with two spurs. Tip of hind tibia (Fig. 29 g) with a very long, stout and barbed spur, 120 μm, and a comb composed of 12 free spines, but without the short spur present in most other species of Orthocladiinae. Tips of middle and hind tarsi I and II each with two subterminal spurs. Pulvilli absent. fLR 0.58; mLR 0.42, hLR 0.43 (both very small). fTR 0.13, fBR 3.2, mBR 2.4, hBR 3.3.

Setae on abdominal tergites are relatively numerous and distributed almost evenly, 72 on I, 90 on II, 72 on

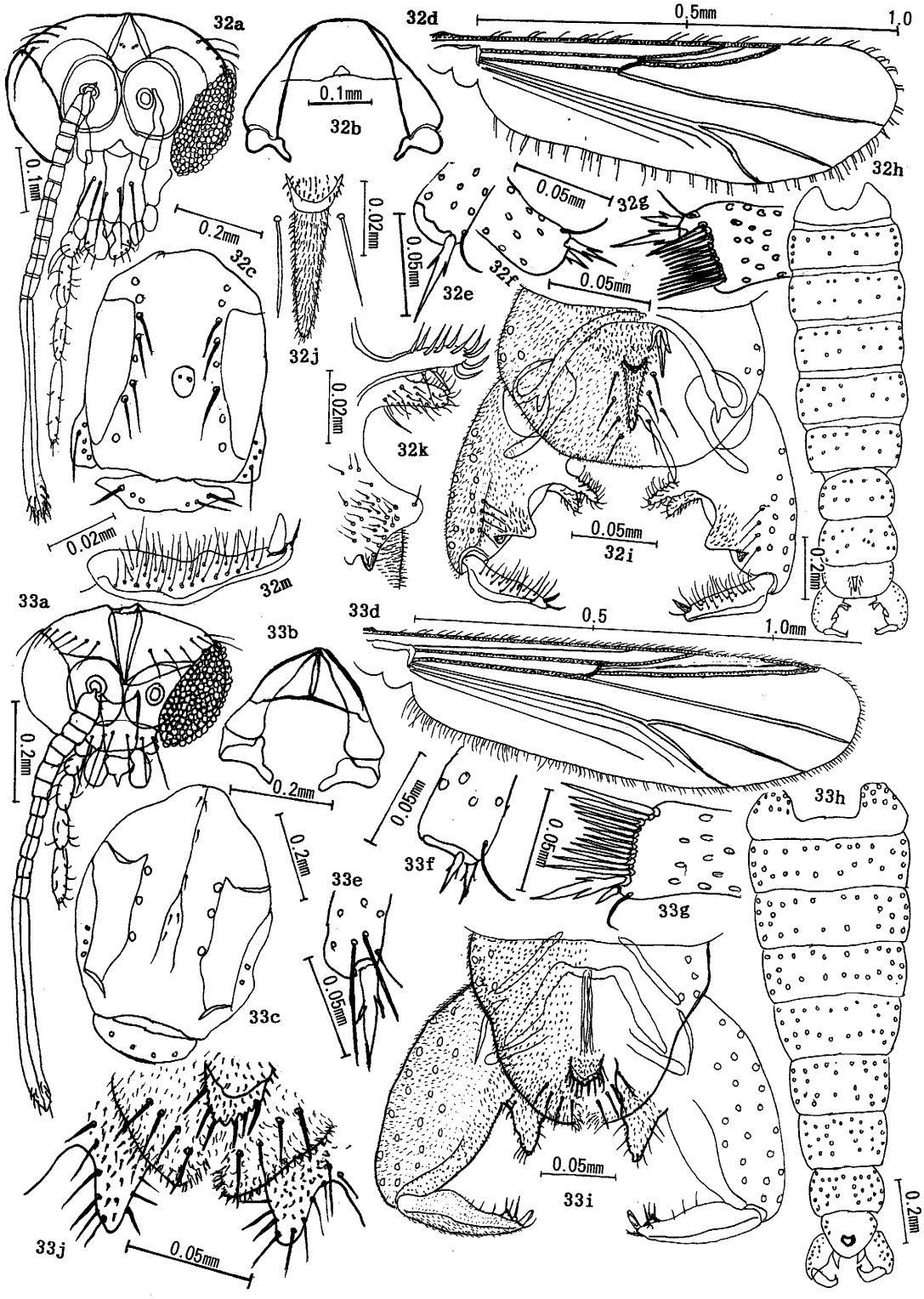

Fig. 32 ***Pseudosmittia mongolzeaea*** **sp. nov.** a: head; b: antepronotum; c: scutum and scutellum; d: winf; e: tip of front tibia; f: tip pof middle tibia; g: tip of hind tibia; h: abdominal tergites; i: hypopygium; j: point; k: inner lobes of gonocoxite; m: gonostylus. **Fig. 33** ***Pseudosmittia mongolzebea*** **sp. nov.** a: head; b: antepronotum; c: scutum and scutellum; d: wing; e: tip of front tibia; f: tip of middle tibia; g: tip of hind tibia; h: abdominal tergites; i: hypopygium; j: anal point and inner lobes.

III and IV, 66 on V and VI, 80 on VII, and 64 on VIII. Hypopygium in Fig. 29 h. Anal point narrow and long, apically pointed. Inner lobe of gonocoxite low and broad. Gonostylus simple, without preapical tooth.

Remarks. This species is considered as belonging to the genus *Metriocnemus* van der Wulp, 1876, since the basic structures are same as those of the subfamily Orthocladiinae, and wing with macrotrichia, eyes bare, Cu2 nearly striaght, costa extending beyond tip of R4+5, and anal point is present (Sasa and Kikuchi, 1995). It is most closely related among the European species to *M. gracei* Edwards, 1929, in that wing with macrotrichia on entire surface, gonocoxite without inner lobe, and anal point is long, but in the latter AR is only about 0.5, anal point in more robust, and gonocoxite with a preapical tooth (cf. Pinder, 1978, Fig. 131C). On the other hand, it is closely related to a Japanese species *M. oiraquintus* Sasa, 1991, described as based on a single specimen collected at the side of the Oirase River, Aomori, northern Honshu, in that narrow anal point is present, tarsi I and II of middle and hind legs with two apical spurs, AR is about 1.0, hLR is 0.43 and very small, and since we cannot detect any essential difference from it, the present specimen is provisionally identified as belonging to this species.

***Mongolcladius* gen. nov.**

A new genus belonging to subfamily Orthocladiinae. Small species with wing length of about 1.5 mm. Eyes bare, without dorsomedial extension. Antenna with 13 flagellar segments, AR about 0.8. Antepronotum united in the middle, without lateral seta. DM absent, setae on scutum, scutellum and abdominal tergites very small in numbers. Wing bare, membrane granular, costa extending beyond tip of R4+5, which is distinctly proximal to tip of Cu1. Cu2 nearly straight. Squama with fringe hairs. Anal point small, bare, narrow and sharply pointed apically. Inner lobe of gonocoxite hook-like.

This genus is most similar to *Trissocladius* Kieffer, 1908, in that wing is bare, granular and squama is fringed, Cu2 is nearly straight and costa is extending beyond tip of R4+5, pulvilli are absent, and anal point is small, sharply pointed and without lateral setae and microtrichia, but differs from the latter in that hind tibial comb is present (absent in *Trissocladius*), and tip of R4+5 is proximal to tip of Cu1. At present, only one species described here is included.

34. *Mongolcladius mongolwexeus* sp. nov.

(Figs. 30 a-i)

Eight males were collected at River Orkhon (# 43) on August 18, 1996, at 1,775 m high from sea level. Holotype: 311 : 86. Paratypes: 311 : 87-92; 309 : 42.

Male. BL 2.08-2.58 (2.38 in average of 8) mm, WL 1.30-1.52 (1.43) mm, WW/WL 0.34-0.37 (0.35, very wide). Ground color of scutum yellow, stripes brown, scutellum yellow, postnotum brown, legs and abdominal tergites brownish yellow. Head in Fig. 30 a. Eyes bare, reniform and without dorsomedial extension, ER 1.33-1.58 (1.43 in average of 8). Antenna with 13 flagellar segments, AR 0.74-0.85 (0.80), AHR 0.38-0.46 (0.43). Palp short, P/H 0.71-0.89 (0.82). SO composed of 1 inner and 4 or 5 lateral setae. CL all 4. Antepronotum (Fig. 30 b) united in the middle, without lateral setae. Distribution of setae on scutum and scutellum in Fig. 30 c. DM all 0, DL all 6 : 6, excepting in a specimen with 7: 7. PA 2-4 (2.9, most frequently 3), SC all 6, excepting in a specimen with 7.

Wing bare, brownish and finely granular, venation in Fig. 30 d. Squama with 5-10 (7.5) fringe hairs. Costa extending much beyond tip of R4+5, which is proximal to tip of Cu1, R/Cu 0.94-0.97 (0.96). R2+3 ending about midway between tips of R1 and R4+5, RR 0.45-0.53 (0.49). VR 1.13-1.17 (1.15). Cu2 nearly straight, anal lobe obtuse. Tip of front tibia (Fig. 30 e) with a long spur, 49 μm long and 1.6 times as long as the diameter of front tibia at the tip in the holotype. Tip of middle tibia (Fig. 30 f) with two short spurs. Tip of hind tibia (Fig. 30 g) with a long (58 μm) and a short (27 μm) spur, and a comb composed of 14 free spines. Tarsomeres IV cylindrical and longer than V. Pulvilli absent.

Setae on abdominal tergites (Fig. 30 h) are relatively small in the numbers, and are grouped roughly into the lateral and the median groups, 32 on I, 2 on II, 26 on III and IV, 20 on V and VI, 18 on VII, and 16 on VIII in the holotype. Hypopygium in Fig. 30 i. Anal point small, bare, narrow and sharply pointed apically. Ninth tergite without long setae in the middle portion, with 3 or 4 short setae on posterior margin flanking anal point. Inner lobe of gonocoxite single, composed of a rectangular base and an inner finger-like process directed backwards. Gonostylus simple, widest at about middle, without preapical tooth.

Remarks. This species can be differentiated from the previously known species of this group by the characters shown in the description of new genus.

***Mongolyusurika* gen. nov.**

A new genus of the subfamily Orthocladiinae. Small midge with WL of about 1.5 mm, wing narrow, WW/WL 0.27. Eyes bare, reniform. Antenna with 13 flagellar segments, AR 1.6-2.0. Setae on scutum and

scutellum very long. Squama fringed. Wing bare, granular. Cu2 nearly straight. Costa does not extend beyond tip of R4+5. R/Cu >1.0. Anal point absent. Inner lobe of gonocoxite small, with strong setae.

The above characters indicate that it belongs to the subfamily Orthocladiinae, and is most closely related the genus *Chaetocladius* Kieffer, 1911 (Cranston *et al.*, 1989b). However, it differs essentially from the latter in that anal point is absent, setae on scutum are all well developed and unusually long, and mid tibia has one long and one short terminal spurs. At present, only the following species is included.

35. *Mongolyusurika mongolxeyeus* sp. nov.

(Figs. 31 a-k)

Thirteen males were collected at Bogdrhan on August 5, 1995, 2,200 m high from sea level. Holotype: No. 306 : 31. Paratypes: No. 306 : 32-42, 311 : 17, 100

Male. BL 2.76-3.04 (2.85 in average of 8) mm, WL 1.44-1.54 (1.49) mm, WW/WL 0.26-0.28 (0.27, very narrow). Head in Fig. 31 a. Eyes bare, inner margin concave but without dorsomedial extension, ER 0.85-1.16 (0.95). Palp slightly longer than width of head, P/H 1.02-1.15 (1.06). SO 8-12 (10.8), CL 8-12 (9.8). Ante pronotum (Fig. 31 b) united in the middle, with 6-8 (6.6) long lateral setae. Distribution of setae on scutum and scutellum in Fig. 31 c. DM 8-18, all unusually long. DL 18-28 (21.3), very many and all very long. PA 7-10 (8.1), SC 10-12 (10.8), on a transverse line.

Wing bare but conspicuously granular, venation in Fig. 31 d. Squama with 5-8 (6.2) fringe hairs, anal lobe obtuse. Costa extending slightly beyond tip of R4+5, which is slightly distal to tip of Cul, R/Cu 1.02-1.06 (1.04). R2+3 separated, RR 0.36-0.42 (0.40). VR 1.21-1.30 (1.27). Cu2 nearly straight. Tip of front tibia (Fig. 31 e) with a long and barbed spur, 72 μm and 1.8 times as long as the diameter of front tibia at the tip in the holotype. Tip of middle tibia (Fig. 31 f) with a long (48 μm) and a short (26 μm) spur, both barbed. Tip of hind tibia (Fig. 31 g) with a long (60 μm) and a short (30 μm) berbed spur, and a comb composed of 16 free spines in the holotype. fLR 0.56-0.62 (0.59), mLR 0.43-0.47 (0.45), hLR 0.56-0.61 (0.59), fTR 0.13-0.14 (0.14). Tarsi with long beards, fBR 2.6-3.6 (3.2), mBR 3.6-5.6 (4.6), hBR 4.3-4.9 (4.6). Pulvilli absent.

Setae on abdominal tergites (Fig. 31 h) are relatively numerous as midges of this size, and are distributed almost evenly, 60 on I, 50 on II to V, 44 on VI and VII, and 34 on VIII. Hypopygium in Figs. 31 i, J. Anal point absent. Ninth tergite with a broad and rounded lobe bearing 12 marginal setae on posterior margin. Inner lobe of gonocoxite small, rod-like, and bearing 5-7 short but strong setae. Gonocoxite with rather long setae around base of inner lobe. Gonostylus (also in Fig. 31 k) with strongly expanded inner margin and nearly straight lateral margin, megaseta large and stout.

Remarks. This species can be differentiated from those of the related genera by the character indicated in the definition of this new genus. Especially noteworthy are the presence of numerous long setae on scutum, highly granular wing, presence of a long and a short apical spur on middle tibia, absence of anal point, and peculiar structure of inner lobe of gonocoxite.

36. *Pseudorthocladius mongolyezeus* sp. nov.

(Figs. 34 a-i)

A male was collected at River Orkhon (#41) on August 18, 1996. Holotype: No. 309: 43. Scutellum lost from the mounted specimen.

Male. WL 1.76 mm, WW/WL 0.34. Body almost entirely yellow, only scutal stripes brownish yellow. Head in 34 a. Eyes bare, with narrow dorsomedial extension, ER 0.47. Antenna with 13 flagellar segments, AR 1.00, AHR 0.45. P/H 1.00. SO 10:10, CL 10. Antepronotum (Fig. 34 b) narrowly united in the middle, with 5: 5 lateral setae. Distribution of setae on scutum and scutellum in Fig. 34 c. DM 16, all minute. DL 8: 8, PA 5: 5. Wing bare, not granular, venation in Fig. 32 d. Squama with 12 fringe hairs. R2+3 ending about midway between tips of R1 and R4+5, RR 0.47. Costa extending much beyond tip of R4+5, which is slightly distal to tip of Cul, R/Cu 1.04. FCu distal to R-M, VR 1.15. Cu2 strongly curved. Tip of front tibia (Fig. 34 e) with a long spur with many barbs. Tip of middle tibia (Fig. 34 f) with two barbed spurs. Tip of hind tibia (Fig. 34 g) with a long, and a short spur, and a comb composed of 14 free spines. Tarsi without preapical spurs. fLR 0.70, mLR 0.51, hLR 0.60, fTR 0.19, fBR 2.6, mBR 2.9, hBR 3.6. Pulvilli absent (an unusual character as a member of this genus).

Hypopygium in Fig. 34 h. Virga small, composed of 4 codes of 12 μm long. Inner process of gonocoxite (Fig. 34 i) quite peculiar in the structure, composed of the dorsal and the ventral lobes, the former narrow and rounded, and with 6 short marginal setae but without microtrichia, and the latter without setae and thickly covered with microtrichia. Gonostylus simple, without preapical tooth.

Remarks. This specimen is considered as belonging to the genus *Pseudorthocladius* Goetghebuer, 1932, since eyes are bare, wing membrane bare and not granular, squama fringed, costa extending much beyond tip of

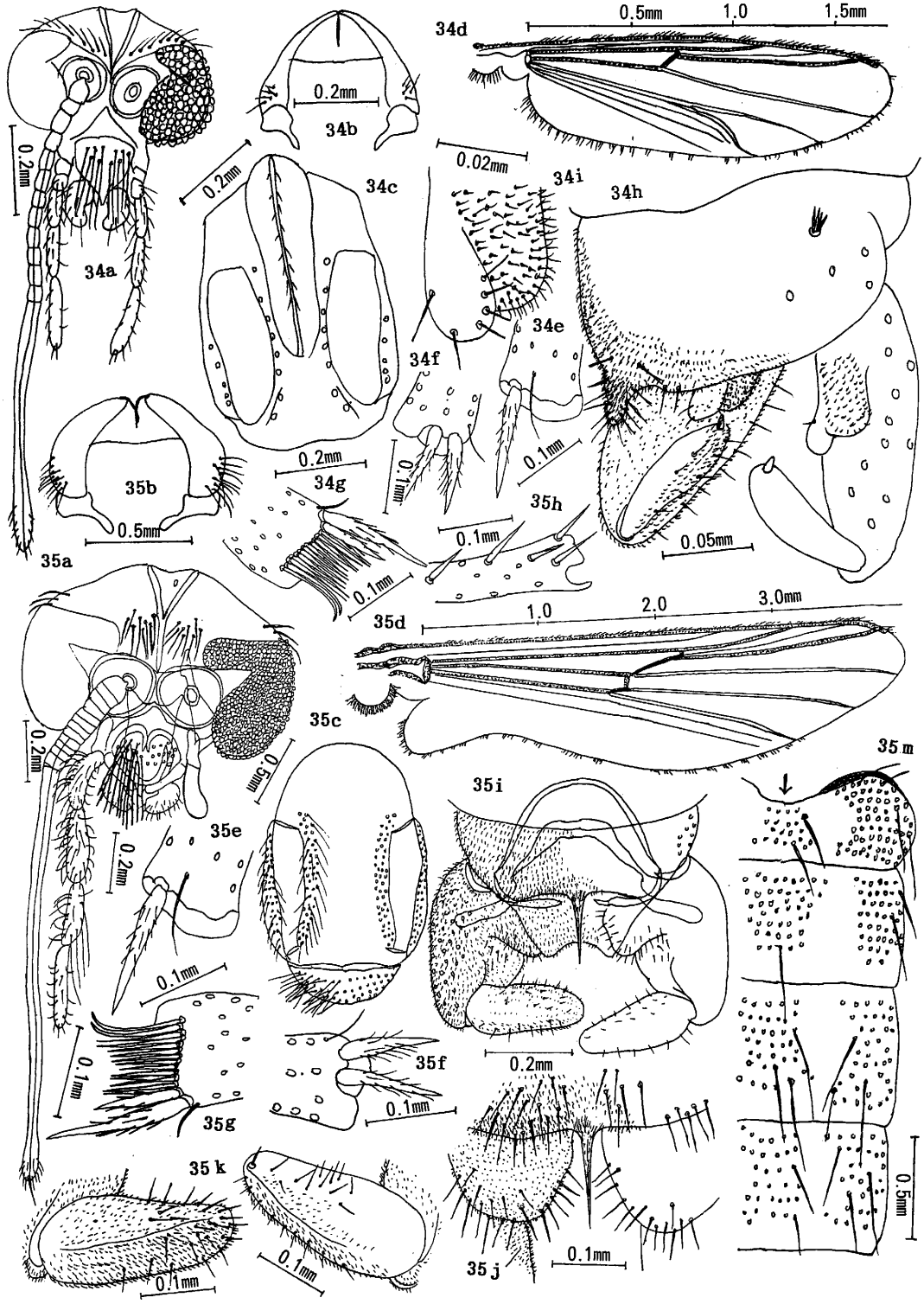

Fig. 34 ***Pseudorthocladius mongolyezeus*** **sp. nov.** a: head; b: antepronotum; c: sutum; d: wing; e: tip of front tibia; f: tip of middle tibia; g: tip of hind tibia; h: hypopygium; i: inner process of gonocoxite. **Fig. 35** ***Pseudodiamesa mongolzecea*** **sp. nov.** a: head; b: antepronotum; c: scutum and scutellum; d: wing; e: tip of front tibia; f: tip of middle tibia; g: tip of hind tibia; h: abdominal tergites I to IV, median and right lateral portions; i: hypopygium; j: anal point; k: gonostylus.

R4+5, Cu2 is strongly curved, and anal point is stout, rounded, covered by microtrichia and with numerous lateral setae (Cranston *et al.*, 1989b). A review of the Holarctic species of this genus was made by Saether and Sublette (1983), and that of the Japanese species by Sasa and Kikuchi (1995). The present species belongs to the group with small virga, anal point well developed and with lateral setae, and gonostylus not expanded and without preapical tooth, and AR is about 1.0, but differs from all the previously known species of this group in the peculiar structure of the inner lobe of gonocoxite, being composed of the double lobes, as stated above.

37. *Pseudosmittia mongolzeaea* sp. nov.

(Figs. 32 a-m)

A male was collected on Mount Bogdrhan, 2,400 m high from sea level, on August 5, 1995. Holotype: No. 306:52.

Male. BL 2.02 mm, WL 1.00 mm, WW/WL 0.34 (very wide). Scutum largely brown and with a central pale hole, scutellum yellow, postnotum dark brown, legs almost evenly yellowish brown, abdominal tergites largely brown and with conspicuous pale bands between the tergites. Head in Fig. 32 a. Eyes bare, reniform and without dorsomedial extension, ER 1.50. Antenna with 13 flagellar segments, AR 1.11. Palp almost as long as the width of head, P/H 1.03. SO 0+3 : 0+3, CL 5. Antepronotum (Fig. 32 b) tapering towards middle and widely separated, without lateral seta. Scutum with a median hole bearing two minute setae, DL 8 : 8, PA 4 : 5, SC 6, as in Fig. 32 c.

Wing membrane bare, brownish, finely granular, venation in Fig. 32 d. Squama bere, anal lobe nearly flat. Costa does not extend beyond tip of R4+5, which is much proximal to tip of Cul, R/ Cu 0.84. R2+3 separated, RR 0.52. FCu much distal to R-M, VR 1.42 (very high). Cu2 strongly curved. Tip of front tibia (Fig. 32 e) with a long and barbed spur, 40 μm long and 1.7 times as long as the diameter of front tibia at the tip. Tip of middle tibia (Fig. 32 f) with two short barbed spurs of much different in length, 25 and 13 μm long. Tip of hind tibia (Fig. 32 g) with a long (39 μm) and a short (18 μm) barbed spur, and a comb composed of 14 free simple spines. fLR 0.48 (unusually small), mLR 0.50, hLR 0.56, fTR 0.14, fBR 3.0, mBR 3.2, hBR 3.5.

The setae on abdominal tergites (Fig. 32 h) are very small in the numbers, 0 on I, 16 on II to VII, and 18 on VIII, and those on II to VII are mostly arranged into the 4 groups, 3 in the lateral, 6 in the anterior, and 4 in the posterior portions. Hypopygium in Fig. 32 i. Anal point (also in Fig. 32 j) long, narrow and apically rounded, 38 μm long and 12 μm wide at the base, situated on a cup with the base on posterior 1/3 of ninth tergite, and entirely covered by microtrichia. Ninth tergite with 4 setae on both sides of anal point. Virga short but prominent, composed of an U-shaped cup and two codes 26 μm long. Gonocoxite with 3 inner lobes (Fig. 32 k, dorsal view), the basodorsal lobe is horn-like, 22 μm long and 4 μm in diameter near the base, and bears numerous short and curved setae. The basoventral one is nearly overlapped by the former, 22 μm long and 14 μm wide at the base, covered by microtrichia. The distal lobe is peculiarly shaped, obtusely triangular and darkly pigmented, 30 μm wide and 12 μm high, covered by numerous short spinelike microtrichia on dorsal side and by longer and thinner microtrichia on ventral side. The third lobe bears short triangular process on posterior margin. Gonocoxite bears 4 peculiar setae arising near the base of the distal lobe. Gonostylus (also in Fig. 32 m) simple, widest at base and tapering towards apex, inner margin straight, without preapical tooth and with numerous very long and fine setae on inner margin.

Remarks. This specimen is very closely related to *P. forcipata* (Goetghebuer, 1921) recorded from Europe in that gonocoxite with 3 inner lobes and the dorsal one is not foot-shaped but broad, and anal point is very narrow, long, and covered by microtrichia, but is regarded as a diffenrent new species because the dorsal lobe is not quadrangular as in *P. forcipata* but is obtusely agulate and highly chitinized, and with a small triangular process on posterior edge, while in *P. forcipata* it is quadrangulate and with a horn-like process on the anterior edge (cf. Pinder, 1978, Fig. 136D). The coloration of abdominal tergites, which are largely brown and with conspicuous pale bands along anterior and posterior margins, is probably characteristic to this species. Such species have not yet been recorded from Japan.

38. *Pseudosmittia mongolzebea* sp. nov.

(Figs. 33 a-j)

A male was collected at Karakorum (# 30) on August 18, 1996. Holotype: No. 309: 44.

Male. BL 2.32 mm, WL 1.27mm, WW/WL 0.30. Scutum, scutellum and postnotum almost entirely brown, only the humeral area and a norrow zone on the midline of scutum yellow. Legs and abdominal tergites almost uniformly yellowish brown. Head in Fig. 33 a. Eyes bare, reniform, ER 1.57. Antenna with 13 flagellar segments, AR 0.98, AHR 0.55. Palp short, P/H 0.80. SO 6 : 6, CL 6. Antepronotums (Fig. 33 b) are united very narrowly in the middle, without lateral seta. Distribution of setae on scutum and scutellum in Fig. 33 c. DM

7, all minute. DL 3:3 (very small), SC 4. Wing bare, venation in Fig. 33 d. Squama bare. Costa does not extend beyond tip of R4+5, which is just above tip of Cul, R/Cu 1.00. R2+3 separated but ending very close to tip of R4+5, RR 0.90. FCu far distal to R-M, VR 1.35. Cu2 moderately curved. Tip of front tibia (Fig. 33 e) with a long barbed spur, 46 μm and 1.6 tiems as long as the diameter of front tibia at the tip. Tip of middle tibia (Fig. 33 f) with two short spurs. Tip of hind tibia (Fig. 33 g) with a long and a short spur, and a comb composed of 12 free spines. fLR 0.41, mLR 0.41, hLR 0.49 (all very small), fTR 0.13 fBR 3.3 mBR 3.8 hBR 5.3

Setae on abdominal tergites (Fig. 33 h) are relatively small in the numbers, 20 on I, 26 on II to VII, and 28 on VIII, and those on II to V are roughly arranged into the anterior and the posterior rows and the lateral groups. Hypopygium in Fig. 33 i. Anal point (also in Fig. 33 j) very small, roughly semicircular, 19 μm high and 17 μm wide, highly chitinized, bearing short marginal setae and microtrichia, and situated on about 1/3 level from posterior margin of ninth tergite. Virga very long and narrow, 50 μm long. Ninth tergite with 8 short setae along posterior margin. Inner lobe of gonocoxite (also in Fig. 33 j) single, longer than wide and acutely angulate. Gonostylus simple, widest at about basal 1/3, with a small rectangular preapical tooth.

Remarks. This specimen is considered as belonging to the genus *Pseudosmittia* Goetghebuer, 1932, since eyes are bare and without dorsomedial extension, squamae are bare, wing membrane bare and finely granular, costa not extending beyond tip of R4+5, Cu2 is curved, and anal point is covered by microtrichia (Pinder, 1978; Cranston *et al.*, 1989; Sasa and Kikuchi, 1995). This species is however quite unusual as a member of this genus in that DM present, tip of R4+5 is not proximal to tip of Cul but is situated just above it, anal point is very small, virga is very long, and inner lobe of gonocoxite is large, longer than wide and acutely angulate.

39. *Smittia aterrima* (Meigen, 1818)

Fourty six males were collected at Mt. Bogdrhan, 2,400 m high from sea level on August 5, 1995. Male: No. 306 : 58-66, 72-75; 309 : 51-55; 311; 18-38, 93-99. Female: 306 : 77. This is a very common species probably distributed all over the world, and have been recorded widely from Europe, and also from a number of localities in Japan by Tokunaga (1940) and by Sasa and coworkers (Sasa and Kikuchi, 1995).

40. *Pseudodiamesa mongolzecea* sp. nov.

(Figs. 35 a-k)

Four males were collected at Bogd (# 5, # 22), about 1,500 m high from sea level, on August 13, 1996. Holotype: No. 309 : 61. Paratypes: 309 : 62-64.

Male. BL 5.84-6.82 (6.40 in average of 4) mm, WL 3.62-4.14 (4.00) mm, WW/WL 0.26-0.28 (0.27). Body almost entirely black, excepting tibiae which are largely yellow and with narrow dark basal and apical rings, and tarsi which are enirely yellow. Head in Fig. 35 a. Eyes bare, each with a long and narrow dorsomedial projection, ER 0.58-0.77 (0.71). Antenna with 13 flagellar segments, AR 3.20-3.88 (3.50), AHR 0.70-0.72 (0.71). Palp long, P/H 1.07-1.16 (1.11). SO composed of 10-14 inner groups and 6-8 lateral groups. CL 20-38 (27.5). Antepronotum (Fig. 35 b) narrowly separated in the middle, without dorsal setae and with 12-16 (14.3) basolateral setae. Distribution of setae on scutum and scutellum in Fig. 35 c. DM all 0, DL 33-44 (38.4), all arising from large pale pits. PA 24-38 (31.5), SC 44-94 (54.0).

Wing bare, bluish, very finely granular, venation in Fig. 35 d. Anal lobe very strongly produced inwards, squama with 104-120 fringe hairs. Costa extending beyond tip of R4+5, which is distal to tip of Cul, R/Cu 1.14-1.16 (1.15). RR 0.63-0.68 (0.66), FCu proximal to R-M and R-Cu, VR 0.82-0.84 (0.83). Cu2 nearly straight. Tip of front tibia (Fig. 35 e) with a long and finely barbed spur, 133 μm long and 1.4 times as long as the diameter of front tibia at the tip. Tip of middle tibia (Fig. 35 f) with two barbed spurs, 96 and 100 μm long. Tip of hind tibia (Fig. 35 g) with a long (136 μm) and a short (75 μm) spur, and a comb composed of 14 free spines. Tarsomeres I and II of front leg, and I, II and III of middle and hind legs each with two simple subterminal spurs. In addition, middle portions of mid tarsomere I with 8, II with 1 and hind tarsomere I with 12, II with 4 simple spurs of similar structue in the holotype (Fig. 35 h, distal half of mid tarsomere I). Tarsomeres IV are cylindrical and slightly longer than V, pulvilli absent. fLR 0.67-0.71 (0.69), mLR 0.46-0.48 (0.47), hLR 0.54-0.56 (0.55), fTR all 0.12. Tarsi with long beards, fBR 4.9-9.4 (7.0), 3.8-4.2 (4.0), hBR 4.7-7.6 (6.2).

Abdominal tergites with large numbers of setae which are arranged roughly into the median and the lateral groups, especially in the first 5 segments (Fig. 35 m, showing bases of the median and the right lateral groups). The total numbers on tergites I to V in paratype No. 309 : 62 are 156 in I, 130 on II, 108 on III, 106 on IV, and 92 on V, with the numbers of the median group of 20, 44, 32, 32, 28, respectively; the abdominal

setae are more evenly distributed on tergites VI to VIII. Hypopygium in Fig. 35 i. Anal point (also in Fig. 35 j, ventral view) is needle-like, very narrow, long, and tapering towards sharply pointed apex, arising from middle portion of ninth tergite. Ninth tergite bears some 10 short setae on both sides of the base of anal point. Inner lobe of gonocoxite (Fig. 35 j) large, rounded and with numerous short marginal setae. Gonostylus (Fig. 35 k; left, dorsal; right, ventral view) elongate oval, apical spur very tiny and thin, seen only from the ventral view.

Remarks. This species is considered as belonging to the genus *Pseudodiamesa* Goetghebuer, 1939 of the subfamily Diamesinae, since cross vein M-Cu is distal to FCu, antepronotum is bare dorsally, and eyes are strongly produced dorsomedially. Reviews of this subfamily were made by Saether (1969) and Oliver (1989). Among the previously known species of this genus, it is very similar in the structure to *P. arctica* (Maloch, 1919), an Arctic species illustrated by Oliver (1989, p. 151), in that wing without macrotrichia and anal lobe is strongly produced inwards, anal point is very narrow, long and sharply pointed, and apical spur (megaseta) of gonostylus is very narrow and tiny, but differs from the latter in that anal point is not parallel-sided but tapering towards pointed apex, ninth tergite without a pair of lobes on posterior margin flanking anal point, inner lobe of gonocoxite is much higher and with rounded margin, and gonostylus is stouter and not tapering towards apex.

41. *Tanypus punctipennis* (Meigen, 1818)

Sixteen males were collected at Bogd (# 8), about 1,500 m high from sea level, on August 13, 1996. No. 309: 66-81. This species has been recorded widely from Europe, and has also been collected from Japan by Tokunaga (1937) and by Sasa and coworkers (Sasa and Kikuchi, 1995).

42. *Procladius crassinervis* (Zetterstedt, 1838)

Two males were collected at Karakorum (# 25) on August 18, 1996. No. 309: 83, 84. This species has been recorded widely from Europe, and also from Japan by Tokunaga (1937), and by Sasa and coworkers (Sasa and Kikuchi, 1995).

DISCUSSION

In view of the recent advances in the studies on the importances of the insects of the family Chironomidae in the environmental and medical sciences, this study is considered as to have contributed to the first step of these problems in the country of Mongol in Central Asia, from which no information on the chironomids have been available. As the results, specimens of as many as 42 species belonging to 23 genera were collected and identified, and only 13 species among them were shown to be in common with those recorded from Europe or Japan, and the rest 29 are described as new species in this paper. These facts suggest that about 1/3 of the species of this family are widely distributed at least throughout the Holarctic Region, but the rest 2/3 have evolved to indigenous species in this rather isolated areas in Central Asia. These evidences are obviously very important contributions to the world taxonomy and bilogy of this group of insects. Furthermore, as the results of this study most of the important chironomid species breeding in Mongolia have become feasible for identification by biological and medical scientists, and the authors hope that the role played by these chironomid species in the environmental and medical fields will be gradually clarified also in this country, and will contribute to the improvements of the health of the Mongolian people.

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Abbreviation: NIES=National Institute for Environmental Studies, Tsukuba, Ibaraki 305. TPEP=Toyama Prefectural Environmental Pollution Research Center, Kosugi, Toyama 939-03.

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TRYPANOSOMA RANGELI: SEM PROFILES DURING THE MIGRATION FROM THE MID-GUT TO THE SALIVARY GLANDS OF A REDUVIID BUG *RHODNIUS PROLIXUS* VIA DORSAL VESSEL

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Abstract: Metamorphosis of *Trypanosoma rangeli* during the migration in the insect host, *Rhodonius prolixius,* was first documented by scanning electron microscopy (SEM). After infection, the parasites reach the insect alimentally tract and then they penetrate into the mid-gut and eventually emerge in the hemolymph of the abdominal cavity. From the abdominal cavity, both the parasites and the hemolymph are propelled to the thorax by the dorsal vessel (aorta pump), the end of which is terminated very near the salivary glands. Thus, once these parasites invade the abdominal cavity, they finally appear in the salivary glands via the transport by the dorsal vessel. The parasites gradually change themselves in shape during the migration. In the present study, we focused on the morphological characteristics of *T. rangeli* at individual stage in the vector by SEM and revealed some new findings in morphology and confirmed a migration route of this species. These results suggest that Guatemalan *T. rangeli* is mainly transmitted through the salivary gland.

Key words: *Trypanosoma rangeli,* insect vector, circulatory system, hemolymph, parasite ultrastructure, SEM

INTRODUCTION

The prevalence frequency of *Trypanosoma rangeli* Tejera, 1920 was from 6 to 10 times or more than of *T. cruzi* in certain regions of Central America (Sousa, 1972). This parasite was first detected in humans in Guatemala (De Leon, 1949) and later found in some reduviid vectors and wild reservoirs (Barrett and Oliveira, 1977). It is not easy to differentiate the species among the flagellar forms of trypanosomes in vectors (Vallejo *et al.,* 1988). Several immunological trials for differtiating species have failed because of cross-reactivity (Anthony *et al.,* 1979; Lopez *et al.,* 1981; O'Daly *et al.,* 1994). However it is necessary to distinguish between the pathogenic and non-pathogenic trypanosomes for epidemiological investigations. On this context, some trials were performed by using various biological methods to distinguish *T. cruzi* from *T. rangeli,* as seen in the reports of Anthony *et al.* (1981), Marinkelle *et al.* (1985), Vallejo *et al.* (1993) and Mello *et al.* (1995). Especially, the last one has reported remakable defferences that *T. rangeli* in the homocoel of *R. prolixus* multiplied very rapidly, while *T. cruzi* showed no division and disappeared eventually from the homocoel of the vector.

Though several controversial data have been observed regarding to the transmission routes, namely, either salivary route or fecal one (D'Alessandro, 1963; Anez, 1982; Hoare, 1972) and whether *T. rangeli* persisted intracellularly as the amastigote form or not in the insect gut (Tobie, 1961; Anez, 1983; Hecker *et al.,* 1990), nontheless it was commonly accepted that this proto-

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zoan parasite was pathogenic to its vector insect (Schaub, 1994; Molyneuz, 1983).

The present paper reports the metamorphism of *T. rangeli* during the migration from the mid-gut through the abdominal cavity and dorsal vessel to the salivary glands of *R. prolixus*. The morphological alteration of the parasite in the mid-gut, hemolymph and salivary glands were traced by a scanning electron microscopy (SEM).

Materials and Methods

Insect and parasite

A laboratory colony of *R. prolixus* which was originally obtained in Zacapa, Guatemala, C.A. in 1993 and maintained thereafter was used. Parasite was of Guatemalan *T. rangeli* strain recently isolated from the salivary gland of *R. prolixus*.

Infection and Dissection

Young nursing mice of the White Swiss strain were infected with *T. rangeli* by injecting the contents of the salivary gland of *R. prolixus* into the peritonium. When high parasitemia appeared about 7 days after infection

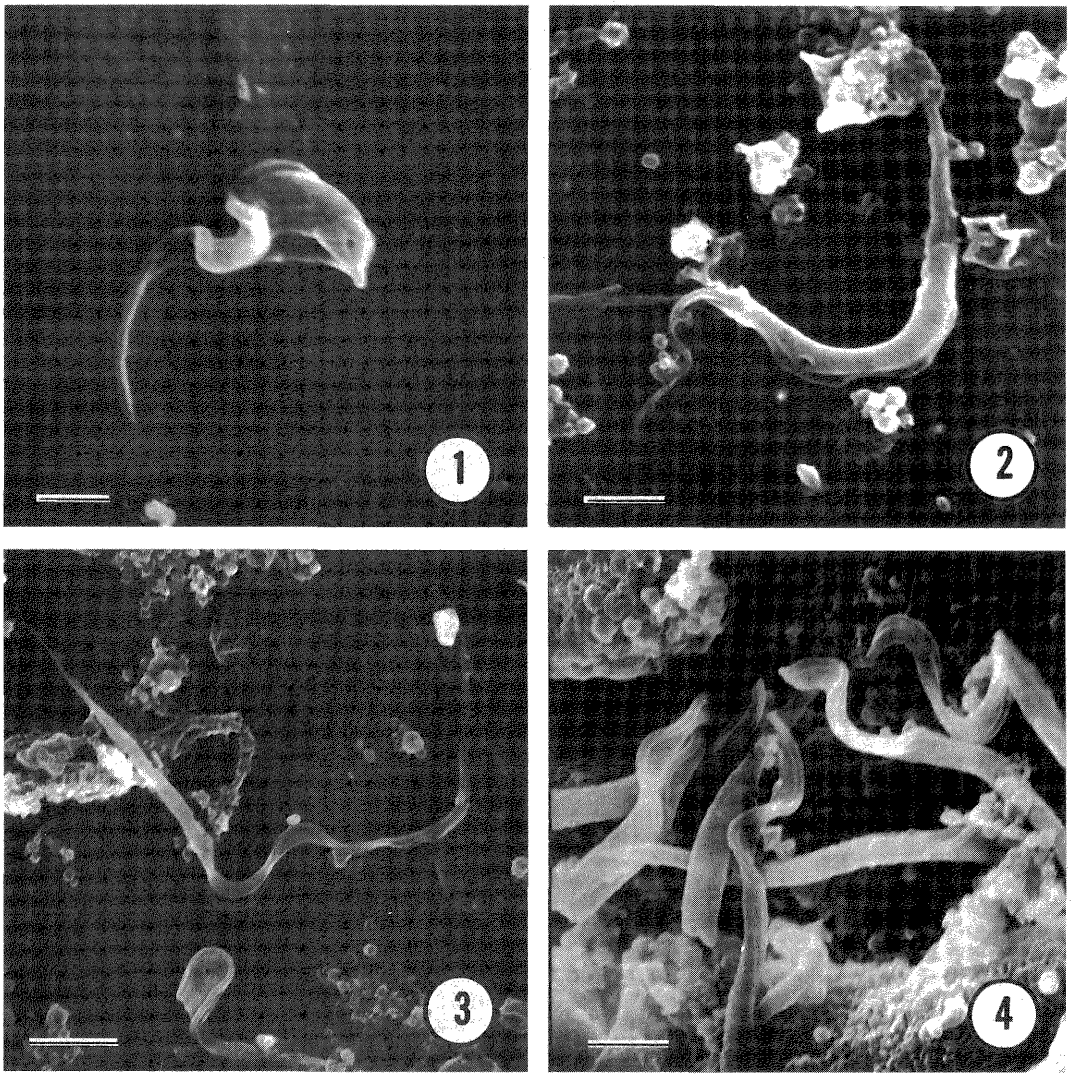

Figure 1 *Trypanosoma rangeli* metacyclic trypomastigote in the salivary gland. Bar=1 μm.
Figure 2 Trypomastigote of *T. rangeli* from the hemolymph. Bar=2 μm
Figure 3 Long epimastigote of *T. rangeli* from the hemolymph. Bar=3 μm
Figure 4 Massive penetration of epimastigotes of *T. rangeli* in the slender midgut. Bar=1 μm

in the mice, various stages of *R. prolixus* reared in laboratory conditions were fed on the blood of infected mice.

Periodically the infected triatomine bugs were dissected, and the hemolymph, salivary glands and gut contents were examined under a light microscopy. The heavily infected bugs were processed for SEM examination. Concurrently Giemsa's stained smear specimens of various tissues were prepared at pH 6.5 for 2 hrs.

Material preparation for SEM

Dissected tissues were washed in saline solution and fixed over night in 3% glutaraldehyde in 0.1 M sodium cacodylate buffer, washed 1 hr in phosphate buffer and postfixed in osmium tetroxide in 2 hours. They were then dehydrated in a graded series of ethanol, dried at critical point in a Hitachi HCP dryer, coated with gold in a JEOL ion-spatter coater, and examined under JEOL U-3 scanning electron microscope at 15 kv.

In addition, the hemolymph and the excrement were placed on a round cover glass previously coated by poly-L-Lysine solution and then dried. The glass was gently placed and attached on the top of a stub. The materials

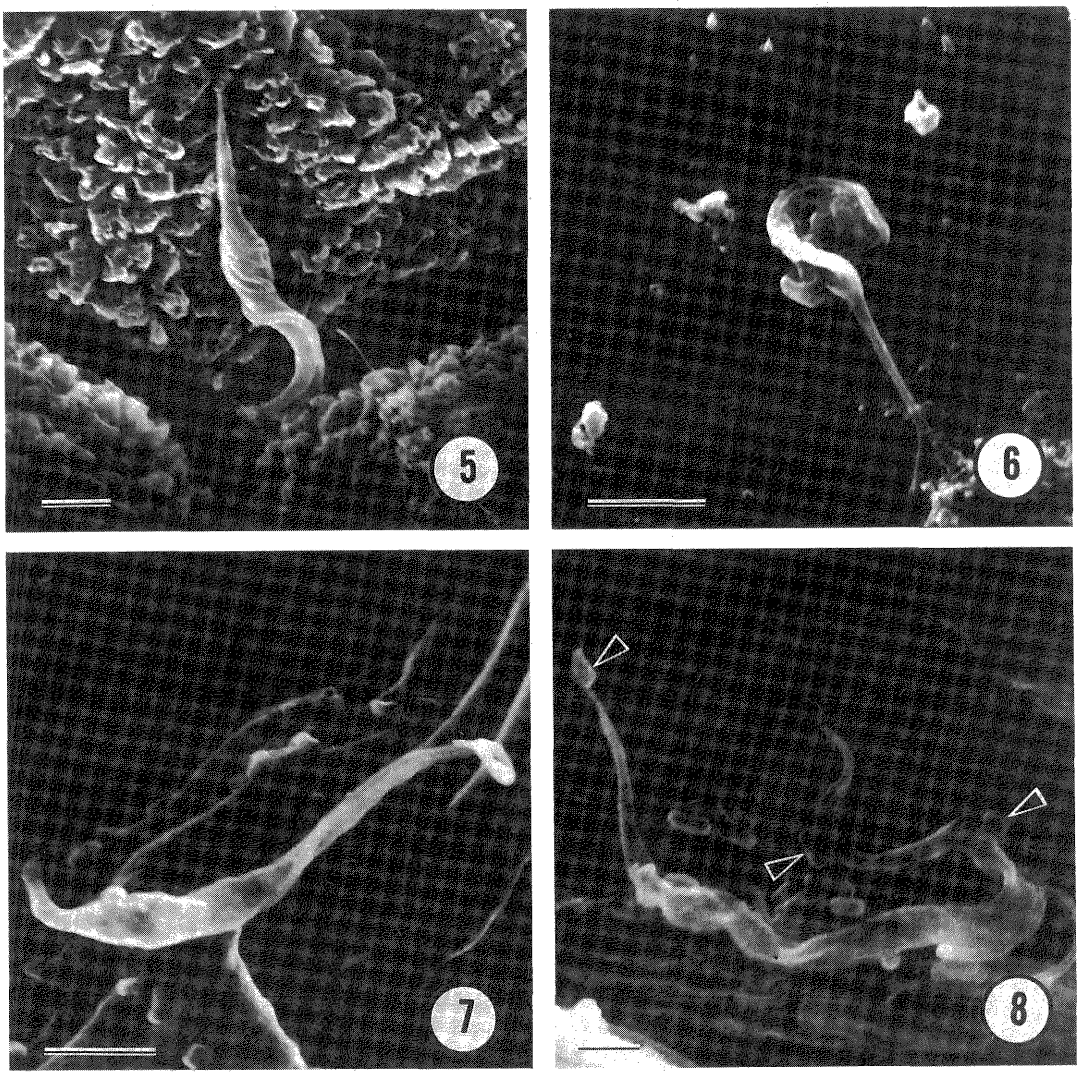

Figure 5 Final phase of penetration to the mid-gut of the host. Posterior portion of epimastigote of *T. rangeli* is evident. Bar=2 μm

Figure 6 Spheromastigote of *T. rangeli* in the vector's gut. Bar=2 μm

Figure 7 Epimastigote of *T. rangeli* in the dorsal vessel of *R. prolixus*; the contractile elements and striated muscle fibers were seen. Bar=2 μm

Figure 8 Epimastigote of *T. rangeli* lying in the dorsal vessel attached with some bacteria (arrowheads) which are commonly found in the gut of the vector. Bar=1 μm

were allowed to stay on the glass for 1 hr and were then fixed with glutaraldehyde. Post-fixation by osmium tetraoxide was omitted before SEM examination, because these small parasites often disappeared from the tissues of the insect by the conventional SEM specimen preparing procedure.

RESULTS

The flagella in all stages of *T. rangeli* were clearly seen in insects. There were three shapes in trypomastigotes in the insect, one in the feces, another in the hemolymph and the last one in the salivary gland. Fig. 1 showed the metacyclic trypomastigote from the salivary glands. The parasite from this gland is the smallest in all and somewhat stout. On the contrary, the trypomastigotes in the abdominal cavity were bigger and slender (Fig. 2), and very similar to those in the feces. The trypomastigotes in the feces had't infectious ability to mice in experimental infection.

Epimastigotes were dominant in the insect hosts. Their bodies were also short, medium and very long in size. However the trypomastigotes distinguished from the epimastigotes by their shape and size. They often segregated in a same host.

Fig. 3 showed one of the longest epimastigotes in the mid-gut.

Fig. 4 showed that the medium sized epimastigotes which were penetrating into the gut epithelium. Fig. 5 showed the last step of the penetration of epimastigote to the mid-gut. As seen in this figure, the body penetration elicited intense spiral movement which was accelerated with its flagellum. This flagellum movement seemed to play a very important role in the penetration. The trigger of the invasion is unknown. Fig. 6 shows the spheromastigote that is developing from the amastigote (which means a round form parasite in the mig-gut epithelium or hemocyte described by Hecker *et al.*, 1990) and is just shifting to epimastigote. This spheromastigote is also a very small in size.

Fig. 7 also showed the epimastigote on the contractile fibers of the folded muscle tissues in the dorsal vessel. Once the epimastigotes intrude into the host's abdominal cavity filled with hemolymph, multiplication occurs there and eventually a great number of parasites is able to be found in the cavity. The dorsal vessel of the bugs absorbs the hemolymph into the aorta which acts as the pumping organ. By this means, the vessel soaked up the parasites together with the liquid and conveyed to the thorax region. As the vessel is terminated very near to the salivary glands in the thorax, the parasites consequently seemed to have penetrated into the salivary glands by themselves. Fig. 8 shows the parasite surrounded by some bacteria (arrowheads) that were probably brought with the parasite from the intestine of the insect into the vessel.

DISCUSSION

Some authors reported that *T. rangeli* trypomastigote from feces could infect mice (D'Alessandro, 1963; Hoare, 1972), while the other noted that only the parasites from the salivary glands had infectivity (Tobie, 1961; Anez, 1982). Present study showed that there were some differences in shapes and sizes of the trypomastigotes and epimastigotes in various regions of the vector insect, and this rapid morphological changes are related to this parasite infectivity. In mice infection with trypomastigotes from the salivary glands, we always saw parasitemia, whereas we could not see it with the parasites from the bug excrements. On the other hand, mice infected with the parasites from the hemolymph, no parasitemia occurred. These results agreed with the findings of Tobie (1961) and Anez (1982). From these findings, we want to suggest that the salivary glands play an important role for the transmission of *T. rangeli* to mice. It is reported that there is a difference in the tissue affinity in bugs between *T. rangeli* and *T. cruzi*; *T. rangeli* favors to live in the slender mid-gut, whereas *T. cruzi* prefers the rectal sac in the same host. Furthermore *T. cruzi* attached to the rectal wall tightly (Zeledon *et al.*, 1984), while *T. rangeli* crawled freely in the mid-gut. In addition, the epimastigotes of *T. rangeli* in the hemolymph and the dorsal vessel were provided with very long and slender body, which is one of the characteristics of this species.

For these characteristcs mentioned above, we facilitated the differentiation of each species by careful separation of the vector's hemolymph, salivary glands, rectal sac and the slender mid-gut.

Spheromastigotes have been reported in both species of *T. cruzi* and *T. rangeli* (De Lucena *et al.*, 1973; Vallejo *et al.*, 1988; Marinkelle *et al.*, 1985) as a shifting stage from amastigote to epimastigote.

Present study is the first report for documenting spheromastigote form of *T. rangeli* by SEM.

It has been described that in *T. rangeli* infection, the symbionts (some bacteria) were small in number in the hemolymph of the bugs (Schaub, 1994). In the present study, however, we observed many bacteria in the hemolymph and even in the circulatory system of the vector by SEM. This is probably because of the tissue

damage of the insect gut due to massive invasion of the parasites.

In this study, trypomastigotes in the salivary glands as well as some stages of epimastigotes and spheromastigotes of *T. rangeli* in *R. prolixus*, the vector were first demonstrated by SEM. The authors here emphasize that flagella of trypanosomes seem to play a major role in the penetration to the gut wall.

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NUCLEOTIDE AND DEDUCED AMINO ACID SEQUENCES OF THE WHOLE STRUCTURAL PROTEINS AND NS1 NONSTRUCTURAL PROTEIN OF FOUR DENGUE 4 VIRUS STRAINS, ISOLATED FROM PATIENTS IN BANGKOK, THAILAND, WITH DIFFERENT CLINICAL SEVERITIES

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Abstract: All structural protein genes as well as one of the nonstructual protein NS1 gene of four dengue virus serotype 4 (DEN 4) strains recently isolated from patients admitted to the Children's Hospital in Bangkok, Thailand in 1993, were sequenced and analyzed. Isolates from a dengue fever (DF) case, CT93-77, dengue hemorrhagic fever (DHF) grade I cases, CT93-129 and CT93-158 as well as DHF grade II case, CT93-74 were selected in this study. It was found that the DHF grade II isolate had the highest percentage sequence divergence in the PrM and M regions from the published prototype strain (Caribbean, 814669). In all four Thai isolates, the C region had the lowest divergence, while the PrM region had the highest divergence both in nucleotide and amino acid sequence. In the whole analyzed regions, the percentage divergence ranges from 8.02-8.33% (nucleotide) and 3.97-4.90% (amino acid) respectively. Three amino acid replacements were found to be specific to the DF strain : at position 136, phenylalanine (F) instead of leucine (L); at position 914, phenylalanine (F) instead of serine (S) and at position 1,042, glutamine (Q) instead of histidine (H). The mean hydrophobicity and isoelectric point value did not show significant differences in the sequenced region for all analyzed isolates. When compare to other strains from different geographical locations, four amino acid replacements were found unique to the Thai isolates. These were at position 269, isoleucine (I) instead of valine (V) ; at position 374, methionine (M) instead of valine (V); at position 482, threonine (T) instead of lysine (K) and position 608, threonine (T) instead of alanine (A). Phylogenetic analysis using the M/E junction showed that DEN 4 from different geographical areas can be classsified into three genotypic groups, and the Thai isolates were divided into two genotypes.

Key words: dengue virus type 4, Thai strain, sequence, virulence, genotype, mutation, clinical severity

INTRODUCTION

Dengue viruses belong to the monquito-borne *flavivirus* causing significant morbidity and mortality of children in tropical countries (Westaway *et al.*, 1985a; Wengler 1991). Dengue virus infection may be asymtomatic or may lead to illness in humans, varying from a mild form of dengue fever (DF) up to the severe form of dengue hemorrhagic fever (DHF) (Nimmannitya, 1987), which can progress to a sudden and fatal hypovelemic shock called dengue shock syndrome (DSS) (Nimmannitya *et al.*, 1969, Allen, 1992).

The disease is caused by 4 dengue virus serotypes (DEN 1, DEN 2, DEN 3 and DEN 4). Dengue viruses are positive sense, single stranded RNA viruses possessing sequence of 5'-C-PrM/M-E-NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5-3' like other *flaviviruses* (Westaway *et al.*, 1985b; Castle *et al.*, 1986; Rice *et al.*, 1986, Coia *et al.*, 1988). Each of the dengue serotypes shares common antigens with other *flavivirus* (Shope and Sather, 1979; Monath *et al.*, 1986), which is supported by genetic sequence data (Block *et al.*, 1989; Chu *et al.*, 1989; Trent *et al.*, 1989). Dengue virus presents a serious health problem in many tropical and sub-tropical countries

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including the Americas, Africa, Asia, Australia and Pacific Islands (Gubler, 1996), especially causing great medical and public health problems in Southeast Asia (Halstead, 1980; Halstead, 1992).

Each serotype of dengue virus may have strains that vary in virulence (Rosen, 1977, 1986). It is presently unknown whether specific virus genotypes are responsible for the more severe forms of the disease or for particular widly spread epidemics. Seroepidemiological studies suggest that severity of DHF/DSS may result from immune enhancement in the secondary infection by preexisting antibodies produced by preceding heterotypic infection (Halstead, 1981, 1988), or by maternal antibodies in the primary infection (Burke, 1988). There is another possibility that the severity of diseases may be related to molecular differences of genomic structure associated with the virulence of a particular strain (Duangchanda *et al.*, 1994; Thant *et al.*, 1996).

The main objectives of this study is to determine the nucleotide sequence of the whole structural (C, PrM/M, E) and NS1 gene of recent isolates of DEN 4 in Thailand, and to correlate the disease severity to the observed molecular differences.

MATERIALS AND METHODS

Virus strains

Four isolates of DEN 4 were selected out of 9 strains from the previous study (Chanyasanha *et al.*, 1995). These isolates were obtained from patients sera with different clinical manifestations at Children's Hospital, Bangkok, Thailand in 1993. Written consent was obtained from the patient's parents or guardians and the study protocol was approved by the ethical review research committee, established by the Ministry of Public Health, Thailand. Isolate (CT93-77) was from a dengue fever (DF) case, 2 isolates (CT93-129 and CT93-158) were from dengue hemorrhagic fever (DHF) grade I cases and another isolate (CT93-74) was from DHF grade II case (Table 1). Clinical severity grading and clinical diagnosis were classified according to the World Health Organization (WHO) criteria (Anonymous, 1986). The viruses were isolated by inoculation of patients sera into *Aedes albopictus* clone C6/36 cell line (Igarashi, 1978) and serotypes were identified by reverse transcriptase polymerase chain reaction (RT-PCR) using type specific dengue primer pairs (Morita *et al.*, 1991; Tanaka, 1993). They were then stored at −80°C. The virus isolates were amplified again by growing in C6/36 cells, at 28°C for 7 days before use.

Table 1 Clinical manifestation of patients from which four selected DEN 4 isolates were isolated

Clinical manifestation of the patients	Dengue 4 isolates
DF	CT93-77
DHF Grade I	CT93-129, CT93-158
DHF Grade II	CT93-74

Preparation of viral genomic RNA

The total genomic RNA was extracted from the infected tissue culture fluid by a single step guanidinium thiocyanate method (Ausubel *et al.*, 1991) with slight modifications. Briefly, 200 μl of infected tissue culture fluid was added to 500 μl GSM solution (5M guanidinium-thiocyanate, 25mM sodium citrate, 0.1M 2-mercaptoethanol) and vortexed shortly, followed by a quick spin. One hundred microlitres of 2M sodium acetate (pH 4.0), 500 μl water saturated phenol and 100 μl chloroform : isoamyl alcohol (49:1) were added and mixed by vortexing. The mixture was then incubated on ice for 30 minutes and centrifuged at 15,000 rpm for 20 minutes at 4°C. The pellet was then washed with 500 μl 75% ethanol, air dried and resuspended in RNase-free 10 μl of sterile distilled water and stored at −80°C until use.

Primer designed for amplifying target gene

The primers for amplifying the target regions were designed with one pair for each of the C, PrM and M gene region covering 839 bases. The E protein gene, which is 1,482 bases long, was divided into 3 regions of approximately 600 bases each. The NS1 gene which is 1,230 bases long was divided into 2 regions of approximately 700 bases each. Both sense and complementary primer pairs were constructed for each region according to published sequences (Zhao *et al.*, 1986; Mackow *et al.*, 1987). Oligonucleotide primers were synthesized using an Applied Biosystem DNA synthesizer (Model 392, California, USA). A schematic diagram of nucleotide sequencing strategy and DEN 4 primers for C, PrM/M, E and NS1 are shown in Figure 1. Sequences covering the whole structural and nonstructural NS1 protein were determined from cDNA clones of six overlapping gene regions (Table 2). The above mentioned primers were used to amplify the gene regions for each of the four selected strains of DEN 4 virus.

Amplifying the target gene region by RT-PCR and purification

For complementary DNA (cDNA) synthesis, 10 μl of RNA samples were incubated at 65°C for 5 minutes

Table 2 Nucleotide sequences of DEN 4 primers used in this study

Code	Sequence (5′-3′)	Position	Gene region
D4-54S *	AAC-ACA-GOO-CTA-ACA-GTT-T	54-72	C, PrM, M
D4-959C †	CCT-ACT-CCT-ACG-CAT-CGC-AT	959-940	
D4-42S	GGA-AGC-TTG-CTT-AAC-ACA-GT	42-61	C, PrM, M
D4-1160C	ACA-TCT-TGT-TGC-CGT-AGT-TA	1160-1141	
D4-878S	AAC-AGG-AAT-CCA-GCG-AAC-TG	878-897	E
D4-1484C	TGT-TAG-TTC-TCC-ATA-GTC-CG	1484-1465	
D4-1376S	AGA-CAC-CCA-TGC-AGT-AGG-AA	1376-1395	E
D4-1978C	ACT-TTT-TCC-TTG-TTT-FHF-TH	1978-1959	
D4-1831S	CAT-ACA-CGA-TGT-GTT-CAG-GA	1831-1850	E
Di-2517C	TGT-ACT-GTT-CTG-TCC-AAG-TG	2517-2498	
D4-2370S	TGC-ATA-GCT-GTT-GGA-GGA	2370-2387	NS1
D4-3031C	CTC-TCT-CAA-TAA-CCC-AT	3031-3012	
D4-2258S	AAG-TGT-GTA-TAC-AAC-CAT-GT	2258-2277	NS1
D4-3031C	CTC-TCT-ATC-CAA-TAA-CCC-AT	3031-3012	
D4-2906S	GTT-CAC-GAC-CAA-CAT-ATG-G	2906-2924	NS1
D4-3674C	AHH-AGA-CAT-AGT-GTC-CCC-C	3674-3656	

* S: Sense, † C : Complementary

and mixed with 10 μl of RT-mixture containing Moloney murine leukemia virus reverse transcriptase (M-MLV-RT) 1 μl (200 U/μl), 5X first strand buffer (Gibco BRI) 4 μl, 0.1M dithiothreitol (DTT) 4 μl, deoxynucleoside 5'-triphosphates (dNTPs) 0.5 μl (10-mM/μl), RNase inhibitor 0.5μl (10 μmole/μl) and complementary primer 0.5 μl (50 pmol/μl). The mixture was incubated at 37°C for 30 minutes. Five microliters of cDNA product were used for PCR reaction. A PCR mixture of 45 μl composed of 10X TTH buffer 5 μl, dNTPs 0.5 μl (10 mM/μl) *Thermus thermophilus* (Tth) polymerase 0.25 μl (4 U/μl), distilled water 37.5 μl, sense 0.5 μl and complementary primer 0.5μl (50 pmol/μl) was added to the cDNA product. The PCR reaction was carried out for 35 cycles using a thermocycler (Mini cycler TM, MJ research, USA) with 94°C denaturation for 1 minute, 53°C annealing for 1 minute and 72°C primer extension for 1 minute.

The PCR products of each region from each DEN 4 isolate was purified by agarose gel electrophoresis and subsequently extracted using the Gene Clean II Kit (BioRad, GL 1131-05, Bio101, La Jolla, California, USA). Purified samples were electrophoresed in 3% agarose gel to confirm the DNA band, and the purified DNA was kept at −20°C until used.

Cloning and sequencing of dengue 4 cDNA

The purified cDNA was ligated into pCR™ II vector using TA Cloning Kit (Ivitrogen, USA) and was used to transform JM-109 or XL-1 blue *Escherichia coli* (Hanahan, 1983). Plasmids from the transformant colonies were isolated by the boiling method (Sambrook *et al.*, 1989) and insertion was comfirmed by *EcoRI* enzyme digestion and PCR amplification using a pair of target primers. After confirmation, target recombinant clones were grown in 5 ml of Lubria-Bertani (LB) broth medium supplemented with Ampicillin overnight, and the plasmid DNA containing inserted gene was purified with QIAprep Spin Miniprep Kit (QIAGEN, Germany) and used for sequencing.

Each cDNA target region was sequenced in both directions, using primer, 5'-GGA-AAC-AGC-TAT-GAC-CAT-G-3' as a sense primer and primer, 5'-CCC-AGT-CAC-GAH-GTT-GT-3' as a complementary primer by the dideoxy chain termination method (Sanger *et al.*, 1997), using the DNA sequencing ready reaction kit (Applied Biosystems, USA). Cycle sequence was done in a GeneAmp PCR system 2400 machine (Perkin Elmer, USA) and was carried out at 25 cycles (10 sec at 95°C for denaturation; 5 sec at 50°C for annealing and 4 min at 60°C for extension). The fluorescent labelled PCR products was purified using CENTRISEP COLUMNS (Princeton Separations, Inc; USA) and sequenced by 373A DNA sequencer machine

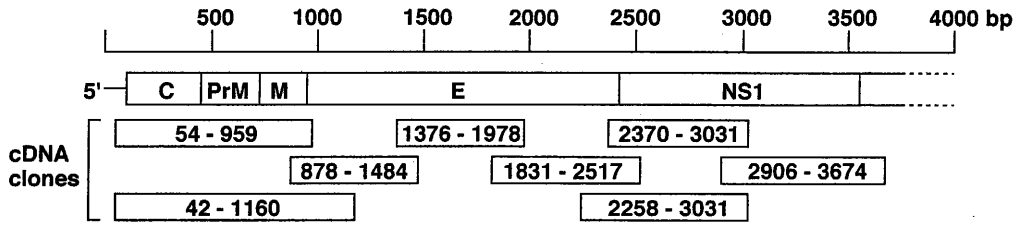

Figure 1 Schematic diagram showing the sequencing strategies of the four Thai DEN 4 isolates. The cDNA clone in each region of C, PrM/M, E, and NS1 are shown in open boxes with nucleotide numbers.

(Applied Biosystems, USA). To avoid sequence variability, 3 different clones from each amplified fragments were isolated and sequenced.

Nucleotide and protein sequence data analysis

Homology comparison and analysis of nucleotide and deduced amino acid sequences were carried out for all 4 strains and compared with published sequences of DEN 4 Caribbean strain 814669 isolated in Dominican Republic 1981 (Zhao *et al.*, 1986; Mackow *et al.*, 1987), with a DNASIS-MAC Version 2.4, NEW CD3 Software System (Hitachi Software Enginerring Co., Ltd., Tokyo, 1994). The nucleotide sequence of the PrM/E junction and E region from other DEN 4 strains was obtained from GEN BANK, NCBI. Phylogenetic analysis was carried out with the aid of the PHYLIP package software (Felsenstein, 1993). The statistical reliability of the phylogenetic tree were assessed using the bootstrap method, with 100 replications (Felsenstein, 1985). The sequence from a representative of dengue serotype 3 virus was used as an outgroup to confirm reliability of the phylogenetic analysis (data not shown).

RESULTS

Comparison of nucleotide sequences between recent isolates of four DEN 4 Thai strains and the prototype strain

The nucleotide sequences of four new DEN 4 Thai isolates and the published strain (Caribbean 814669) are shown in Figure 2. The C region begins at position 1 until 339, followed by PrM, M, E and NS1 regions at position 340 to 612, 613 to 837, 838 to 2,319 and 2,320 to 3,552 respectively. All four newly sequenced Thai isolates revealed nucleotide sequence homologous to the prototype strain, with the base replacements occurring mostly on the third codon resulting in silent mutations. The nucleotide sequences in the PrM region of the four Thai isolates had the highest divergence from the prototype strain ; ranging from 9.84 to 11.36%, followed by the M and NS1 regions, with the divergence range of 8.44-9.33% and 8.03-8.52%, respectively. The C region is the most conserved as shown in Table 3.

Comparison of amino acid sequences among the four DEN 4 isolates and the prototype strain

The deduced amino acid sequences of four DEN 4 isolates, compared to the prototype strain (Caribbean 814669) are shown in Figure 3. The C region begins at position 1 to 113, followed by PrM, M, E and NS1 regions at positions 114 to 204, 205 to 279, 280 to 773, and 774 to 1,184 respectively. In all four isolates, the deduced amino acid sequences exhibited high homology to the prototype strain. However, amino acid replacements at certain position were also observed. When the percentage difference in each region of the four isolates were compared with the prototype strain, highest amino acid variation was found in the PrM (6.59%) region, followed by the NS1 (6.32%), E (4.45%), M (4.40%) and C (1.17%) regions.

Comparison of amino acids among four Thai DEN 4 isolates in relation to the clinical severity of the disease

Specific amino acid replacement among four Thai isolates in relation to the clinical severity of the disease are shown in Table 4. The DHF grade II strain had specific amino acid replacements at positions 119, 145, 207, 470, 520 and 598. The DF strain was shown strain-specific amino acid replacements at position 136 having phenylalanine (F) instead of leucine (L), at position 914 having phenylalanine (F) instead of serine (S) and at position 1,042 having glutamine (Q) instead of histidine (H).

Comparison of amino acid residues at M/E junction and E region of DEN 4 Thai isolates and other isolates from different location

The amino acid sequence of the four Thai isolates and the other 19 isolates from different geographical locations were compared. The amino acids at position

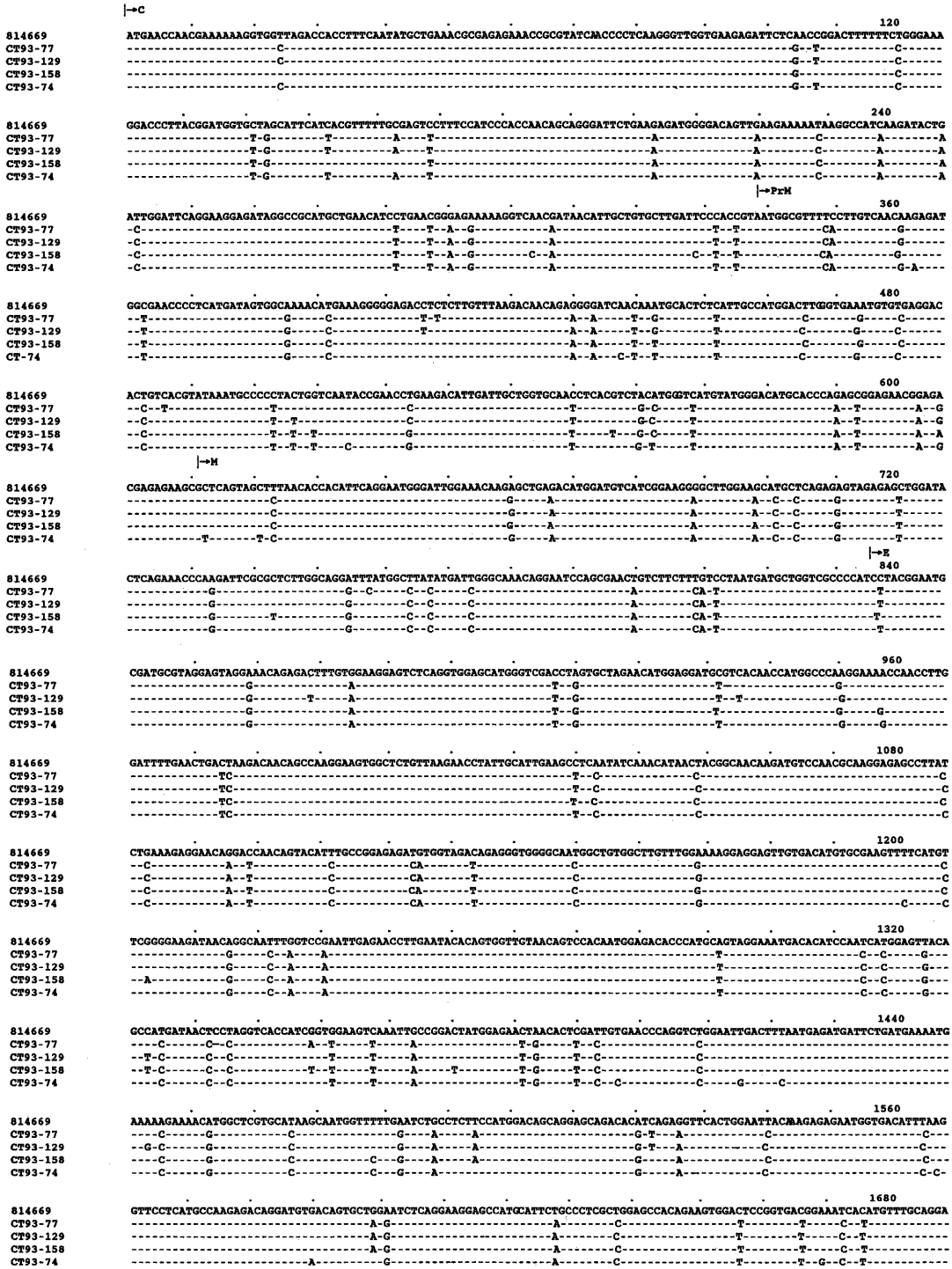

Figure 2 Comparison of nucleotide sequences of the structural (C, PrM/M, E) and nonstructural NS1 protein genes among four Thai DEN 4 isolates and the prototype strain (814669). Nucleotide base positions are numbered beginning from C gene.

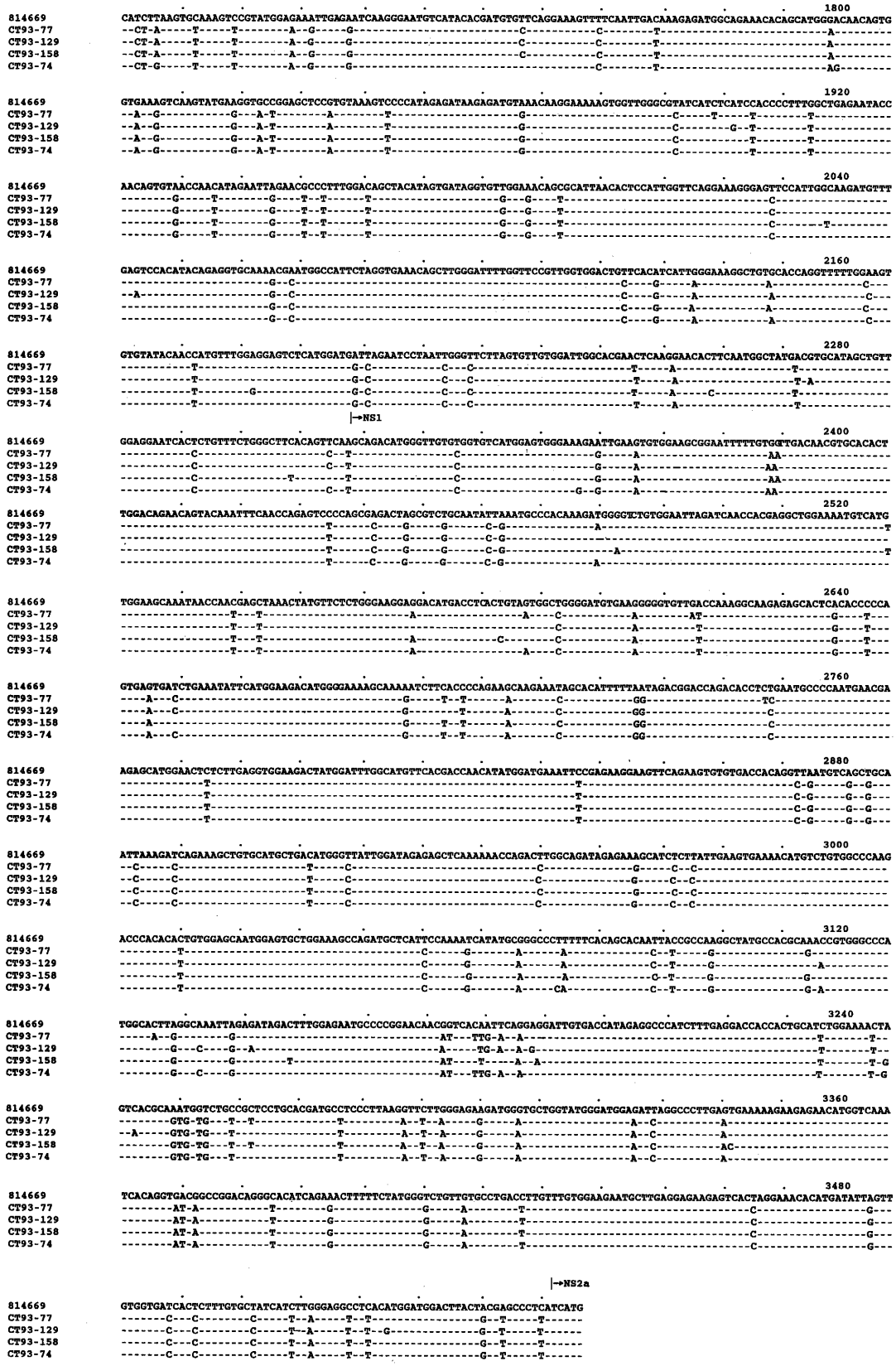

Figure 2 continued.

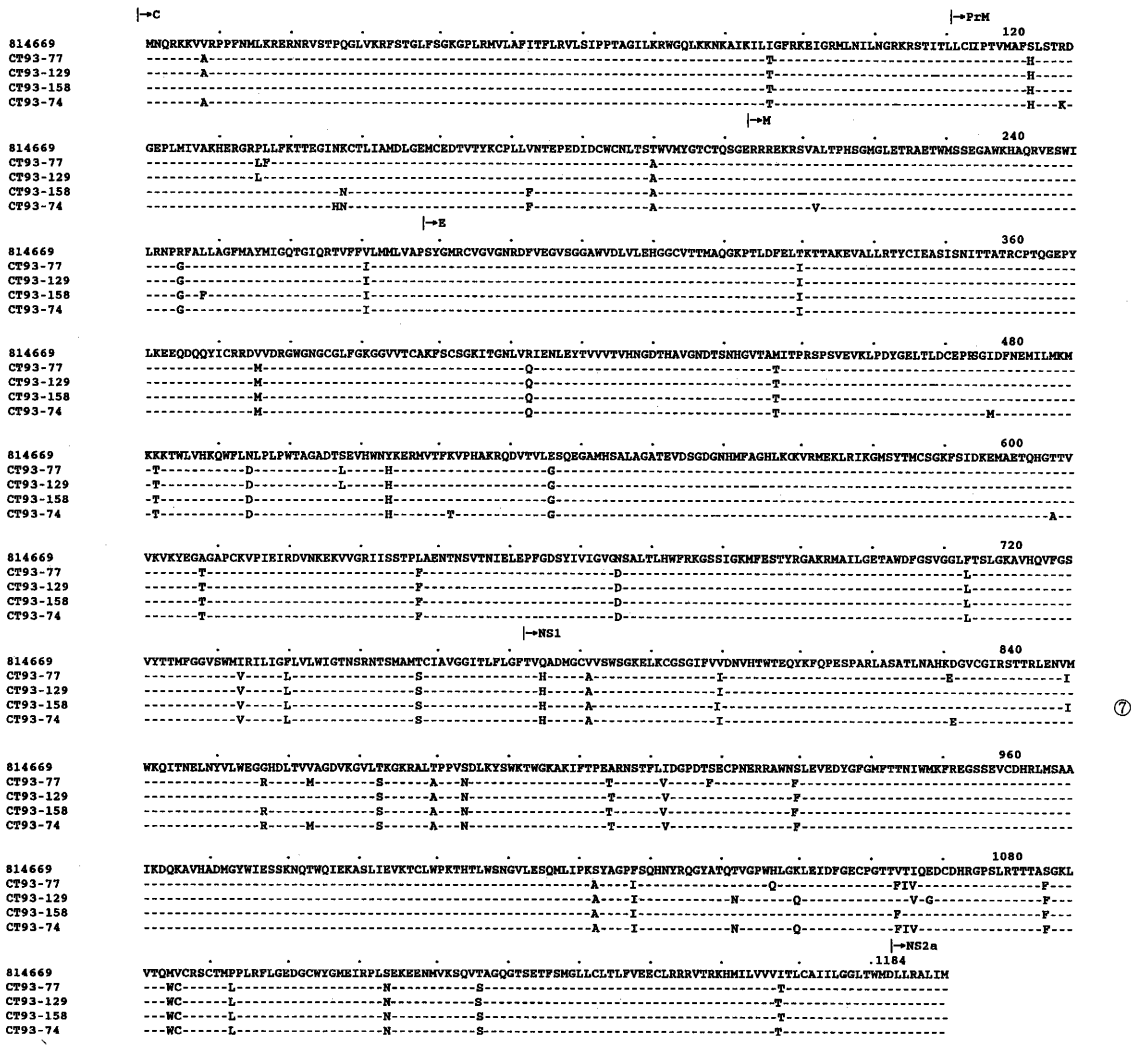

Figure 3 Comparison of deduced amino acid sequences of the structural (C, PrM/M, E) and nonstructural NS1 protein among four Thai DEN 4 isolates and the prototype strain (814669). Amino acid positions are numbered beginning from C gene.

Table 3 Nucleotide and amino acid sequence divergence and percentage of divergence among the four new Thai DEN 4 isolates compared with the prototype strain (814669)

Gene region	Sequence divergence from 814669 strain							
	CT93-77		CT93-129		CT93-158		CT93-74	
	nt * (%)	aa † (%)	nt (%)	aa (%)	nt (%)	aa (%)	nt (%)	aa (%)
C	22 (6.49)	2 (1.77)	22 (6.49)	2 (1.77)	20 (5.90)	1 (0.88)	22 (6.49)	2 (1.77)
PrM	28 (10.26)	4 (4.40)	27 (9.89)	3 (3.30)	28 (10.26)	4 (4.40)	31 (11.36)	6 (6.59)
M	20 (8.89)	2 (2.67)	19 (8.44)	2 (2.67)	20 (8.89)	3 (4.00)	21 (9.33)	3 (4.00)
E	113 (7.62)	20 (4.05)	118 (7.96)	25 (4.05)	119 (8.03)	19 (3.85)	117 (7.89)	22 (4.45)
NS1	105 (8.52)	26 (6.32)	99 (8.03)	21 (5.11)	102 (8.27)	25 (4.87)	105 (8.52)	25 (6.03)
Whole	288 (8.11)	54 (4.56)	285 (8.02)	48 (4.05)	289 (8.14)	42 (3.97)	296 (8.33)	58 (4.90)

* nt : nucleotide, † aa : amino acid

269, 374, 482 and 608 were unique to the Thai isolates. These was at position 269 having isoleucine (I) instead of valine (V), at position 374 having methionine (M) instead of valine (V), at position 482 having threonine (T) instead of lysine (K) and at position 608 having threonine (T) instead of alanine (A) as shown in Table 5.

Table 4 Position of salient amino acid changes from C to NS1 regions of DEN 4 isolates with different clinical severities

Isolates	Clinical severity	Amino acid replacements in								
		PrM			M	E			NS1	
		119	136	145	207	470	520	598	914	1042
CT93-77	DF	R	F	N	A	I	K	T	F	Q
CT93-129	DHF Grade I	R	L	N	A	I	K	T	S	H
CT93-158	DHF Grade I	R	L	N	A	I	K	T	S	H
CT93-74	DHF Grade II	K	L	H	V	D	T	A	S	H

One letter symbol amino acids with abbreviations : A=alanine, D=aspartic acid, F=phenilalanine, H=histidine, I=isoleucine, K=lysine, L=leucine, N=asparagine, Q=glutamine, S=serine, T=threonine, V=valine

Genotype determination of Thai strains from M/E junction and E region

For the genotypic classification of the 7 Thai strains, 1,530 nucleotides (map position 690—2,219) from the M and E regions, were used to construct a phylogenetic tree (Figure 4). The tree showed that the strains analyzed in this study could be divided into three major groups. Group 1 consisted of 1 isolate (TC 2443) from Thailand in 1963, group 2 included isolates from Central America, Brazil, Tahiti and Indonesia and group 3 included the remaining 6 isolates from Thailand, 2 isolates from the Philippines and 1 isolate from Sri Lanka.

DISCUSSION

The nucleotide and amino acids sequences of the entire analyzed genomic regions in four Thai strains in this study differed from prototype strain, ranging equal to or lower than 8.33% and 4.90%, respectively (Table 3). The rates of variation among each strain were similar to the other dengue serotype such as Thai DEN 2 sequences, which were isolated from Maha Sarakham in 1986-1987 (Duangchanda *et al.*, 1994), and Nakorn Phanom in 1993 (Thant *et al.*, 1996), compared with DEN 2 published strains. However, dengue viruses also seem to have lower nucleotide and amino acid change than the other RNA viruses (Buonagurio *et al.*, 1986;

Table 5 Position of salient amino acid changes in M/E junction and E region of DEN 4 isolates compared with other strains isolated from different geographical areas

Strains	Geografical source	Year isolated	M	E		
			269	374	482	608
814669	Dominican Republic	1981	V	V	K	A
1411, 6494	El Salvador	1983, 1994	—	—	—	—
1492	Mexico	1984	—	—	—	—
5489	New Caledonia	1984	—	—	—	—
1650	Puerto Rico	1986	—	—	—	—
1385	Brazil	1982	—	—	—	—
S-44754, 114-094-85	Tahiti	1985	—	—	—	—
S-44750	Sri Lanca	1978	—	—	—	—
30153, 1036, 1132	Indonesia	1973, 1976, 1977	—	—	—	—
H-241, 16589-64, 12123	Phillipines	1956, 1964, 1984	—	—	—	—
TC2443	Thailand	1963	—	—	—	—
D78-01	Thailand	1978	I	—	—	—
D84-024	Thailand	1984	I	—	—	T
CT93-77	Thailand	1993	I	M	T	T
CT93-129	Thailand	1993	I	M	T	T
CT93-158	Thailand	1993	I	M	T	T
CT93-74	Thailand	1993	I	M	T	T

One letter symbol amino acids with abbreviations : A=alanine, I=isoleucine, K=lysine, M=metheonine, T=threonine, V=valine

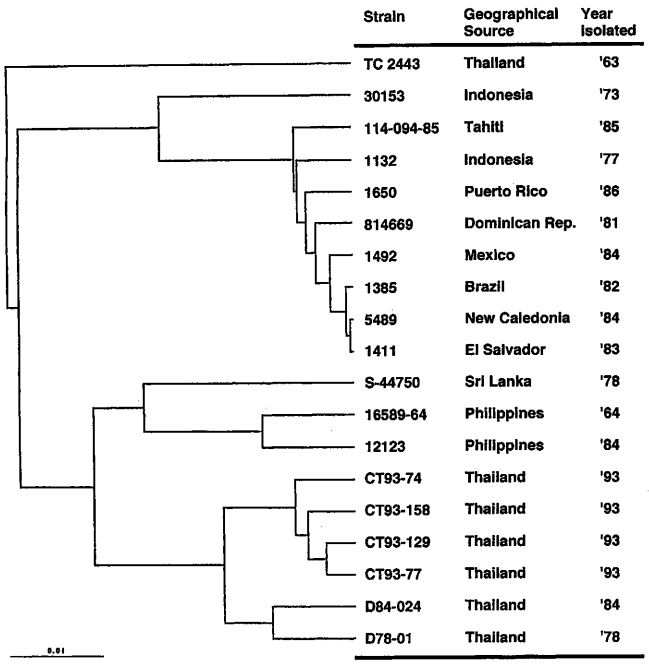

Figure 4 Phylogenetic relationships among 19 DEN 4 strains isolated from different geographical areas. Phylogenetic tree was constructed by comparison of all nucleotide sequences (map position 690 to 2,219). Genetic distance was calculated by DNA dist program. Distance matrix was constructed andphylogenetic tree was deduced by UPGMA method in the PHYLIP package program.

Rico-Hesse *et al.*, 1987; Nichol *et al.*, 1989; Rico-Hesse, 1990; Tanaka *et al.*, 1991; Zheng, 1994; Ali *et al.*, 1995).

Most of the amino acid replacements in the four Thai strains compared with the prototype strain did not alter the nature of the proteins. However there were some amino acid replacements, that could significantly change the nature of the proteins in comparison to the prototype strain : from hydrophobic to hydrophilic : at position 81 (I → T), at 442 (M → T), at 608 (A → T), at 901 (A → T), at 1,085 (V → C), and at 1,163 (I → T). On the other hand, amino acid replacements in the opposite direction from hydrophilic to hydrophobic were found at position 186 (T → A), at 325 (T → I), at 878 (T → A), at 1,019 (S → A) and at 1,077 (S → F) (Figure 3). The amino acid at positions 269, 374, 482 and 608 in M and E regions were found to be unique for the Thai strains (Table 5). These amino acid replacements may have led to variations in the antigenicity.

The mean hydrophobicity and isoelectric point value in each region had been analyzed. Both of this value in each region and whole study region, did not show significant differences (data not shown).

The phylogenetic tree showed that the dengue 4 isolates from different geographical ares segregated into three separate branches, suggesting the presence of three genotypes (Figure 4). Interestingly, two isolates obtained from Indonesia were found to be closely related to the other American strains and the isolate from Tahiti.

There were two main hypotheses concerning the pathogenesis of DHF/DSS. One was that immunopathological mechanisms of hypersensitivity reaction or enhancement of virus replication resulting from secondary infection by heterotype of dengue viruses (Halstead, 1970). On the other hand, several people suggested that virulence of infecting viruses might be related to the severity of the disease (Rosen, 1977). There is also evidence that dengue viruses change genetically and some strains have greater epidemic potential (Gubler *et al.*, 1978; Lanciotti *et al.*, 1994). According to the antigenic and genetic variation of arboviruses which have been described in many studies (Haishi, 1990; Ali and Igarashi, 1997; Thant, *et al.*, 1995), the mechanism which associated with the antigenic strain variation is still not completely understood. It has been proposed that particular amino acid replacements or combinations may lead to antigenic variation and its pathogenicity among strains. The evidence obtained in this study shows that three positions of one DF strain and three DHF Thai strains had strain-specific amino acid replacements at 136 of PrM region (F → L), 914 (F → S) and 1,042 (Q → H) of NS1 regions. These three positions may be critical for severity and are points of interest to be analyzed further by comparing nucleotide and amino acid sequences from various DEN 4 isolates with different disease severity in the same particular time and place.

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Research Note

IN VITRO EFFECTS OF DECOCTION FROM FIVE SPECIES OF CHINESE PLANTS AGAINST *TRICHOMONAS VAGINALIS*

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Abstract: *In vitro* effects of extracts from the 5 species of Chinese herbal medicine against *Trichomonas vaginalis*, one of the most important protozoal diseases in China, were investigated. It was demonstrated that the extracts from *Artemisia annua, Phellodendron amurense* and *Pulsatilla chinensis* were highly effective against the protozoa at 1 to 5 hr after the start of the exposure to the drugs. The extract from *Cnidium monnieri* was much less effective and that from *Sophora flavescens* showed no activity even after the exposure for 5 hr. After this screening for excluding non-effective drugs, the parasites were exposed to all of the drugs except the extract from *S. flavescens*, the negative one in the above test, for 5 days at the drug concentrations lower than those above. The extracts from *A. annua* and *P. chinensis* were highly effective, immobilizing the parasites completely 2 or 3 days after the start of the exposure and afterwards. Contrarily, those from *C. monnieri* and *P. amurense* were not so effective. The results obtained in the present studies were in favour of the prospect that further studies including those on side effects, would be worthy of being carried out for the establishment of the chemotherapy of *T. vaginalis* infection with the crude drugs from medicinal plants in China.

Key words: *Trichomonas vaginalis,* Chinese medicinal plants, therapy

INTRODUCTION

There are a large number of obstinate parasitic diseases still nowadays in Asian and other countries on the earth. Though several excellent drugs have been synthesized for their treatment and control, there still remain matters to be considered. Excellent synthesized drugs are not readily available in local villages because of their high cost and inconvieniences in transportation. This fact might be rather a minor one. However, there is an important problem. Some strains (isolates) of parasitic protozoas and helminths have been found to be insensitive to excellent synthesized drugs (Boreham, 1995; Geerts *et al.*, 1997). For instances, according to Boreham (1995), some lines of *Giardia* resistant to quinacrine and albendazole have been isolated.

The situation with these facts is thought to be where the possible development of new drugs would hopefully come in, based on scientific exploitation of traditional medicinal plants like a global strategy for the control of obstinate parasitic diseases (Maki *et al.*, 1996). This is, at least in part, in accordance with the famous fact that cloroquine-resistant malaria is treated nowadays with quinghaosu, originally a traditional Chinese drug from the plant, *Artemisia annua.*

In this line of thought, the present authors have been testing efficacy of extract from medicinal plants on various kinds of parasites (Maki *et al.*, 1997). Effects of decoction from a number of Chinese plants against *Trichomonas vaginalis* have been one of the topics which interest them.

Much attention has been paid to a large number of Chinese medicinal herbs because of their possible utility for the control of various kinds of parasites (Shen, 1992; Zhao, 1983; Zhao *et al.*, 1991). Traditional medicinal plants, *A. annua, Cnidium monnieri, Phellodendron*

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Table 1 Efficacy in scores of the extracts from the 5 species of Chinese plants against *Trichomonas vaginalis*

Extract diluted (4 to 64 times)	duration of exposure				
	1 hr	2 hr	3 hr	4 hr	5 hr
A. annua					
4	4	4	4	4	4
8	4	4	4	4	4
16	4	4	4	4	4
32	4	4	4	4	4
64	3	4	3	4	4
C. monnieri					
4	1	1	1	1	2
8	1	1	1	1	1
16	0	0	1	1	1
32	0	0	0	1	0
64	0	0	0	0	0
P. amurense					
4	4	4	4	4	4
8	4	4	4	4	4
16	2	4	4	4	4
32	0	3	3	4	4
64	0	2	2	2	4
P. chinensis					
4	4	4	4	4	4
8	4	4	4	4	4
16	4	4	4	4	4
32	4	4	4	4	4
64	3	4	4	4	4
S. flavescens					
4	0	1	1	1	0
8	0	0	0	0	0
16	0	0	0	0	0
32	0	0	0	0	0
64	0	0	0	0	0

Number of *T. vaginalis* moving per field of the view (10× 10) was recorded with the result of more than 50 parasites in control groups. The efficacy of the extracts tested was expressed in scores in this table. The efficacy score 4, 3, 2, 1 or 0 was given to the groups in which the number of moving parasites was 0, 1-5, 6-10, 11-50 or more than 50, respectively.

Table 2 Efficacy of the extract from the 4 species of the plants in the reduction of the number of *T. vaginalis*

Extract diluted (64 to 512 times)	Reduction in %				
	Duration in days after the start of exposure				
	1 day	2 day	3 days	4 days	5 days
A. annua					
64	95	93	100	100	100
128	78	83	72	86	86
256	37	18	56	82	75
512	52	31	5	69	65
C. monnieri					
64	86	84	79	92	88
128	74	72	76	90	85
256	25	41	56	63	59
512	7	37	60	64	57
P. amurense					
64	89	99	83	76	50
128	81	84	70	79	48
256	38	39	58	83	51
512	44	35	69	75	51
P. chinensis					
64	86	100	100	100	100
128	69	98	99	99	84
256	74	65	57	41	23
512	8	39	54	39	33

The mean number of parasites per 10 μl of the medium in the control group with standard deviation was originally recorded. However, statistical analysis was not carried out because the purpose of this study was to find such a decoction at a certain concentration that will prohibit the movement of the parasites completely (see underlined parts).

amurense, Pulsatilla chinensis, and *Sophora flavescens* were chosen for the present study. These medicinal plants are readily available in China, mostly seen in the fields of North-East area of China.

The clinical utility of the 5 plants for therapy of infectious diseases is described in a number of publications including Japanese books. For example, according to Mitsuhashi (1988) and Namba (1993 a, b), these plants are useful for the treatment of parasitic and bacterial infections as follows. *A. annua* (whole herb) is a plant of established utility for the treatment of malaria as mentioned above. Extract from *C. monnieri* (fruits) is described as a drug having anti-*T. vaginalis* action. One of the components of *P. amurense* (bark), berberine has an activity against bacterial infection. *P. chinensis* (roots) containing saponin is useful for the treatment of *Entamoeba histolytica* infection and malaria. And *S. flavescens* (roots) is said to have an anthelmintic action.

It is intriguing to find out a possible efficacy of the extract from the plants including *C. monnieri* against *T. vaginalis*. The present communication describes the comparative effects of the plant extracts against *T. vaginalis* cultured in our laboratory.

Materials and Methods

Decoction tested were as follows. The commercially available medicinal plants in China, *A. annua, C. monnieri, P. amurense, P. chinensis,* and *S. flavescens* were washed and dried. Five grams of the materials was added to 100 ml of distilled water. They were each boiled for 30 minutes. The extract was filtered and preserved as the first part of the drug extraction. Fifty

m*l* of distilled water was added to the residue and this was boiled again. The extract was filtered to obtain the second part of drug extraction. The first and second portion were mixed. The mixed extract was boiled to be concentrated to 10 m*l*. As a result, 10 m*l* extraction was prepared using 5 g of the materials. This decoction was diluted at two folds repeatedly up to nine times with sterilized distilled water. For the test in which the parasites were exposed to the extract for 5 hr, it had been diluted at 4, 8, 16, 32 and 64 folds and for the exposure for 5 days at 64, 128, 256 and 512 folds.

T. vaginalis were donated from School of Medicine, Tokai University, Kanagawa Prefecture, Japan. They were maintained in Asami media containing about 1,500 parasites/m*l* before proliferation at 36.5±0.5°C throughtout the present studies.

A preliminary study was first carried out in order to exclude non-effective drugs among the extracts from the 5 species of the plants. The decoction diluted with sterilized distilled water at a desired concentration (or the water in the controls) and the culture medium containing the parasites were mixed at the ratio of 1:5 throughout the present studies. In the first study, 10 μl of the suspension was taken onto glass slides for their observation at 1, 2, 3, 4 and 5 hr after the mixture had been put into each pore of a plastic plate. Three fields of the light-microscope view (10×10) were checked to count the number of the moving (including "moribund") or immobilized parasites. They were observed sometimes at 10×40 whenever their close examination was necessary. The test was repeated three times for the confirmation of the reproducibility. A special attention was paid as follows to distinguish the moving parasites from the immobilized ones during the preliminary study for 5 hr and the subsequent one for 5 days. The normal parasites like those in control groups were seen rotating actively or going back and forth with the movement of the anterior flagella. The cytoplasm of the normal parasites was clear with transparent granules distributed in the inside. The moribund parasites were attached with more and more materials with the passage of time. The movement of the anterior flagella and undulating membrane became slower and ceased eventually. The cytoplasm of the immobilized parasites contained larger and obscure granules of poor transparency. Their distribution in the cytoplasm was not clear.

The subsequent study was carried out in an attempt to select drugs that immobilize the parasites completely at a certain concentration. In this study, the test tubes containing *T. vaginalis* and the drugs except the decoction from *S. flavescens* in the medium was stood for 5 days. Ten μl media taken was subjected to the observation and count of the moving and immobilized parasites everyday for 5 days. The criterior for the distinction between moving and immobilized parasites was based on the observation above described. The test was repeated three times.

RESULTS

Preliminary study to exclude non-effective drugs

The results of the preliminary tests based on the observation for 5 hr (Table 1) showed that the extract of *A. annua, P. amurense* and *P. chinensis* was highly effective in immobilizing the parasites. These 3 kinds of extraction showed high efficacy against the parasites as early as 1 hr after the start of the exposure. Especially the extract of *A. annua* and *P. chinensis* were found to be extremely effective irrespective of the drug concentrations tested at 1 hr after the start of the exposure. Compared with the efficacy of the extracts from *A. annua* and *P. chinensis*, that from *P. amurense* ramained less effective at lower concentrations of the extract until 3 hr after the start of the exposure. At 4 and 5 hr after the start, there was scarcely any differences among these 3 test materials.

The extracts of *C. monnieri* and *S. flavescens* were found to be much less effective if they had any efficacy (Table 1). At 5 hr after the start of the exposure, the extract from *C. monnieri* seemed to have somewhat effectiveness at 4- or 8-fold dilution. However, no efficacy was detected in the extract from *S. flavescens* under the present experimental conditions.

Thus, the present preliminary study showed that the extracts from all the plants examined except *S. flavescens* had, more or less, an effect immobilizing or killing *T. vaginalis in vitro* following the exposure for 5 hr or less. As the decoction from *S. flavescens* showed no efficacy, this was omitted in the subsequent study.

Examination for finding such drugs that immobilize the parasites completely at certain concentrations

The effects of the extracts from *A. annua, C. monnieri, P. amurense* and *P. chinensis* at comparatively low concentrations were examined for 5 days (Table 2).

The extracts from *A. annua* and *P. chinensis* were found to be highly effective, immobilizing 100% of the parasites at 64-fold dilution, 2 (*P. chinensis*) or 3 (*A. annua*) days after the start of the exposure and afterwards. No complete immobilization was seen under the presence of these extracts at 128-fold dilution or less

concentrations.

The extract from *C. monnieri* was shown to be less effective than those from *A. annua* and *P. chinensis*.

The efficacy of *P. amurense* decoction against *T. vaginalis* was nearly the same as that of *C. monnieri* during 3 days from the start of culture.

DISCUSSION

Infection with *T. vaginalis* was found wherever search was made (Faust *et al.*, 1970). In Japan, 5-10% female and 1-2% male adults were infected with the parasite though the percentage was decreasing (Yoshida, 1992). This sexually transmitted disease, severely harmful to female health, is still nowadays a problem of public-health importance in China. Though the infection rate varies from region to region, one third of females are presumably suffering from the infection with the parasite in some areas of China (Zhang, unpublished). Thus, unfortunately too many people have been infected with this parasite all over the world.

The curative efficacy of metronidazole (flagyl) against *T. vaginalis* is well-known. However, this drug is accompanied with such difficulties that it is rather expensive for many patients as a local standard and is not readily available because of transportation problems as mentioned in INTRODUCTION.

Another way to treat the patients is the use of extracts from medicinal plants. However, this also has a problem to be resolved. The curative efficacy of crude drugs from plants has been obscure and the incomplete treatment of the disease seems to be responsible for another transmission, producing chronic carriers who are very dangerous from the viewpoint of epidemiology.

The aim of the present investigation on the 5 species of Chinese herbal medicine against *T. vaginalis* was to select them scientifically for the development of the new antitrichomonal drugs available even in local areas in China. It is hoped that the topical application of such drugs to patients would eventually control the further transmission of the parasitic disease. Needless to say, such plant-derived drugs would have the possibility of playing an alternative role in the treatment of *T. vaginalis* patients when this parasite is found to be resistant to metronidazole in developed countries and areas.

In both the present preliminary and the subsequent investigation, the extracts of *A. annua* and *P. chinensis* were found to be highly effective against *T. vaginalis*, immobilizing 100% of the parasites under certain conditions. Though extract from *C. monnieri* is described as a drug effective against the disease caused by *T. vaginalis* (Mitsuhashi, 1988; Namba, 1993a), the effectiveness was not high as far as the present study was concerned.

The extract from *P. amurense* was fairly effective in the test of the exposure for 5 hr while it was not so effective in that for 5 days. What was resposible for this is a matter for speculation now. One of the possibilities is that effective components in question are unstable.

Thus, the extracts described as a drug effective against protozoal and bacterial diseases were found to reduce the number of *T. vaginalis in vitro*. However, the extract from *S. flavescens*, thought to be useful in eliminating parasitic helminths (Mitsuhashi, 1988; Namba, 1993a) was not effective against the parasitic protozoa, *T. vaginalis* in the present study.

Based on the data so far obtained, the extracts from *A. annua* and *P. chinensis* are thought to be promising because they could suppress the movement of the parasites completely under certain conditions. However, the possible side effects of the extracts under the conditions still remain to be studied before clinical application.

In conclusion, the present research would be beneficial for the basic study leading to the development of new drugs from the wealth of Chinese medicinal plants which have been believed to be useful from the ancient times.

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Case Report

ASYMPTOMATIC SCHISTOSOMIASIS HAEMATOBIA: AN IMPORTED CASE

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Abstract: Stool and urine examinations revealed eggs of *Schistosoma haematobium* in a 31-year-old asymptomatic Japanese female returned from Africa. She was treated with praziquantel, and good therapeutic result was obtained. However, as *S. haematobium* is not indigeneous to Japan, most Japanese doctors and medical technologists are unfamiliar with this parasitic disease, they need to be reminded of its existence when they encounter persons who have visited or resided in endemic areas, and of the necessity of urine and stool examinations of such persons.

Key words: *Schistosoma haematobium*, schistosomiasis, praziquantel

INTRODUCTION

Schistosomiasis haematobia, a common helminth disease caused by the infection with *Schistosoma haematobium* in certain countries in Africa and western Asia, is a major public health problem in endemic areas (Mahmond, 1988). Although many Japanese now travel to or stay in tropical or subtropical areas where *S. haematobium* is endemic, only a very small number of Japanese patients have been reported in Japan in the 1990's (Yamaguchi *et al.*, 1993; Kobari *et al.*, 1996; Ohnishi, 1997). Recently, we treated an asymptomatic Japanese female patient infected with *S. haematobium* who had recently returned from Africa. The purpose of this report is to emphasize the importance of parasitological investigation for people who have visited or resided in tropical developing countries, even if no symptoms of infection are evident.

CASE DESCRIPTION

A 31-year-old female Japanese stayed in Niger from January 1995 to April 1997 as a member of Japan Overseas Cooperation Volunteers. Subsequently, she traveled in Côte d'Ivoire, Ghana, Togo, Cameroon and Thailand, and she returned to Japan on May 2, 1997. During this period, she came into contact with water from both a river and a pond in Africa. Although she had no symptoms, she was referred to my outpatient clinic on May 2, 1997, because a health examination on her return to Japan had revealed *Entamoeba* cysts in her stool. She was initially treated with oral administration of metronidazole. Eggs of *S. haematobium* (Fig. 1) were revealed by centrifugation sedimentation stool examination on May 8, and urine examination on May 15, 1997, but neither macroscopic nor microscopic haematuria was found. The urine mentioned above was obtained at about 11:00 a.m. Her blood count and serum biochemical tests were normal, except for her blood eosinophil count, which was 750/mm^3 (WBC 9,600/mm^3; 7.8%

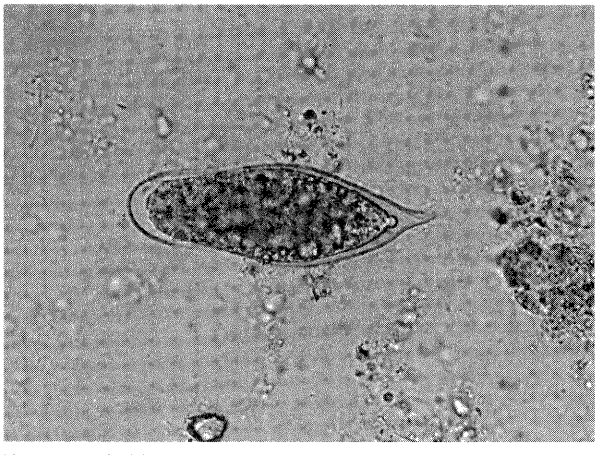

Figure 1 *Schistosoma haematobium* egg with a distinct terminal spine in stool (×380).

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eosinophils) on May 2, 1997. She was diagnosed as having *S. haematobium* infestation, and was treated with oral administration of 30 mg/kg and 20 mg/kg doses of praziquantel on the mornings of and evenings of May 22 and 23, 1997, respectively. Neither side effects nor abnormal laboratory changes due to this drug were found. Urine, which were obtained between 10:30 and 11: 30 a.m. and stool examination on May 29 and August 11, 1997 revealed no eggs of *S. haematobium,* and her blood eosinophil count was 260/mm^3 (WBC 5,200/mm^3; 5.0 % eosinophils) on August 11, 1997.

DISCUSSION

The intermediate hosts of *Schistosoma* spp., several types of snails, discharge cercariae, the larval stage of *Schistosoma* spp., in fresh water. On contact with the skin of humans, cercariae of *S. haematobium* penetrate into the skin, enter the venous circulation, and grow in the intrahepatic portal vessels. Subsequently, they migrate into the rectal veins and vesical and pelvic plexuses, and the female worms produce eggs in the venules (Beaver *et al.*, 1984). Eggs were found in both the stool and urine in my patient, indicating that the female worms may have produced eggs in the vessels of both the lower part of the colon and urogenital systems. Although many Japanese have traveled to or stayed in *S. haematobium*-endemic areas and come into contact with river or pond water, few cases of schistosomiasis haematobia have been reported in Japanese patients. This disease may have been overlooked in many Japanese patients because cases infected with only small number of worms are commonly asymptomatic and Japanese doctors and medical technologists are unfamiliar with this disease. The number of Japanese contracting schistosomiasis haematobia will increase as more people travel to *S. haematobium*-endemic areas. Japanese doctors and medical technologists should be aware of this disease when they encounter people who have returned from such areas, and of the necessity of the urine and stool examinations of these people.

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