Morphological and Habitat Differences Between Two Forms of Japanese *Limnodrilus hoffmeisteri* Claparède (Oligochaeta, Tubificidae)[1]

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(*With 7 Text-figures and 2 Tables*)

A tubificid oligochaete, *Limnodrilus hoffmeisteri* Claparède is one of the commonest zoobenthos in various fresh- and brackishwaters of the world. This species is generally distinguished from other congeneric species by having chaetae with subequally bifurcate teeth and penis sheaths with moderate length (300–700 μm). Since these specific characters varies considerably (e.g. Brinkhurst, 1963; Kennedy, 1969; Dzwillo, 1984; Steinlechner, 1987), however, several forms of uncertain taxonomic status have been recognized so far (Brinkhurst, 1965; Brinkhurst and Jamieson, 1971; Hiltunen, 1967; Barbour *et al.*, 1980; Bird and Ladle, 1981; Ohtaka, 1985).

In Japanese waters, too, one of us (A.O.) discriminated the following two forms of *Limnodrilus hoffmeisteri* on the basis of difference in penis sheaths (Ohtaka, 1985; also see, Brinkhurst and Jamieson, 1971):

"Typical" form: Worms with "typical" type of penis sheaths; a "typical" type of penis sheath is a somewhat bent sheath with the hood set at right angle to the shaft, and the lumen turning about 90° (Fig. 3A, B).

"Plate-topped" form: Worms with "plate-topped" penis sheaths; a "plate-topped" type of sheath is a straight sheath with a plate-topped hood that sometimes has a scalloped edge (Fig. 4A, B).

Although he treated these two forms as infraspecific variations (Ohtaka, 1985), a more detailed study is needed to elucidate their taxonomic status. In the present paper, we examine chaetal and penial morphology and geographical

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distributions of these two forms.

Materials and Methods

Specimens used in the present study were collected from 55 localities, cover-

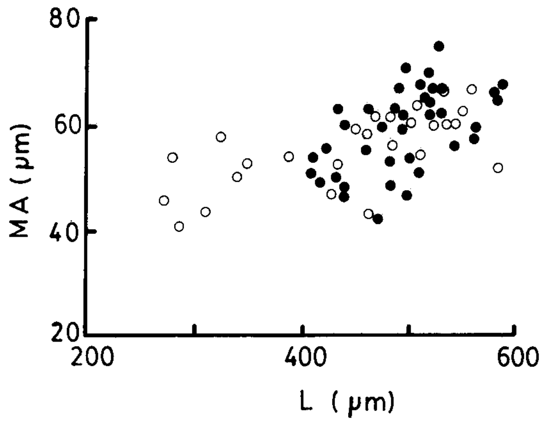

Fig. 1. Correlations between the length (L) and maximum diameter (MA) of penis sheath in *L. hoffmeisteri* from R. Arakawa. Solid circles, specimens with spermatozeugmata; open circles, other stages of mature specimens.

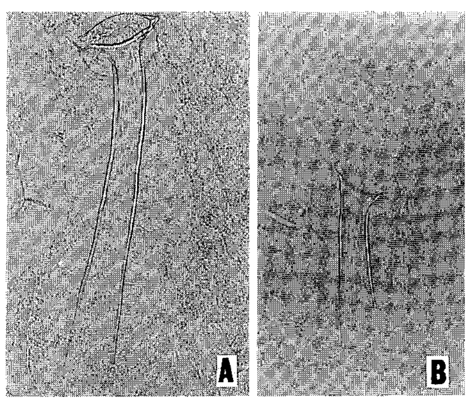

Fig. 2. Developing or degenerating penis sheaths of *L. hoffmeisteri*. Their lengths are shorter than those of complete sheaths, and both proximal and distal ends of the shafts are fade out. Specimens from M. Sakata, Niigata (A) and R. Gobou, Takamatsu (B).

ing various types of freshwaters in Japan. They includes all the specimens used in Ohtaka (1985) and are listed in the appendix at the end of this paper.

The following values were measured or calculated on a part of collected specimens (the numbers of specimens examined are given in Table 1);

1) Ratio of distal chaetal teeth (=upper tooth length [UT]/lower tooth length [LT]). This ratio was measured on chaetae in either of 5th to 8th segments because it was usually consistent within individuals at least in these segments.

2) Length of penis sheath (L), and minimum diameter (MI, measured at the nec) and maximum diameter (MA, measured at the proximal end) of penial shaft, and ratios L/MI, L/MA and MA/MI. Only the specimens having spermatozeugmata were used to compare penis sheaths, since penis sheaths of these apparently mated and reproductively active specimens less varied than those in other mature ones (Fig. 1). This is probably because the latter included developing or degener-

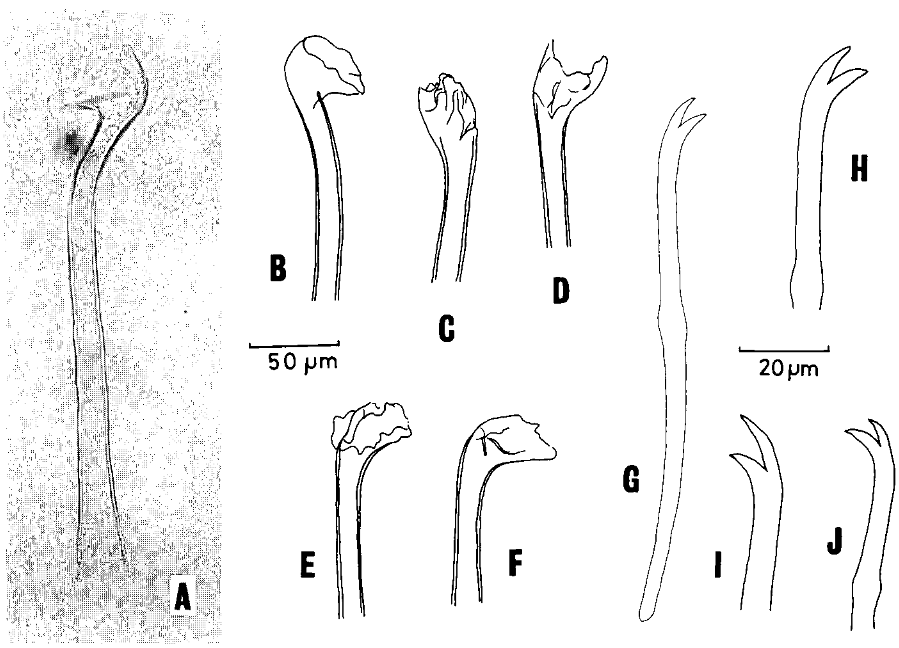

Fig. 3. Penis sheaths and chaetae of "typical" form of Japanese *L. hoffmeisteri*. A-F, penis sheaths: A, B, F, normal "typical" types (A, L. Touro; B, L. Shikotsu); C-E, clumped sheaths (L. Kuttara, E and F are a pair from one specimen). G-J, cheatae: G, H, normal chaetae (R. Shinkawa, Sapporo); I, J, variations (I, L. Okotanpe; J, southwestern shore of L. Ō-numa). A and G show entire forms; others only distal ends.

ating penis sheaths (Fig. 2) in addition to normally developed ones. Brinkhurst and Wetzel (1984) also stressed that the critical identification of *Limnodrilus* species needed appropriate use of mated specimens.

Observations and measurements were made under an optical microscope on specimens mounted in either of Canada balsam, synthetic resins (Bioleit®, Ohken Ltd., Tokyo, or Eukit® O. Kindler, Freiburg), Amman's lactophenol (Brinkhurst, 1963), or polyvinyl lactophenol, on glass slides with cover slips. Polyvinyl lactophenol according to Jones (1946) was improved as follows:

6.3 g	polyvinyl alcohol
40 ml	distilled water
45 ml	lactophenol (lactic acid: phenol = 1 : 1)

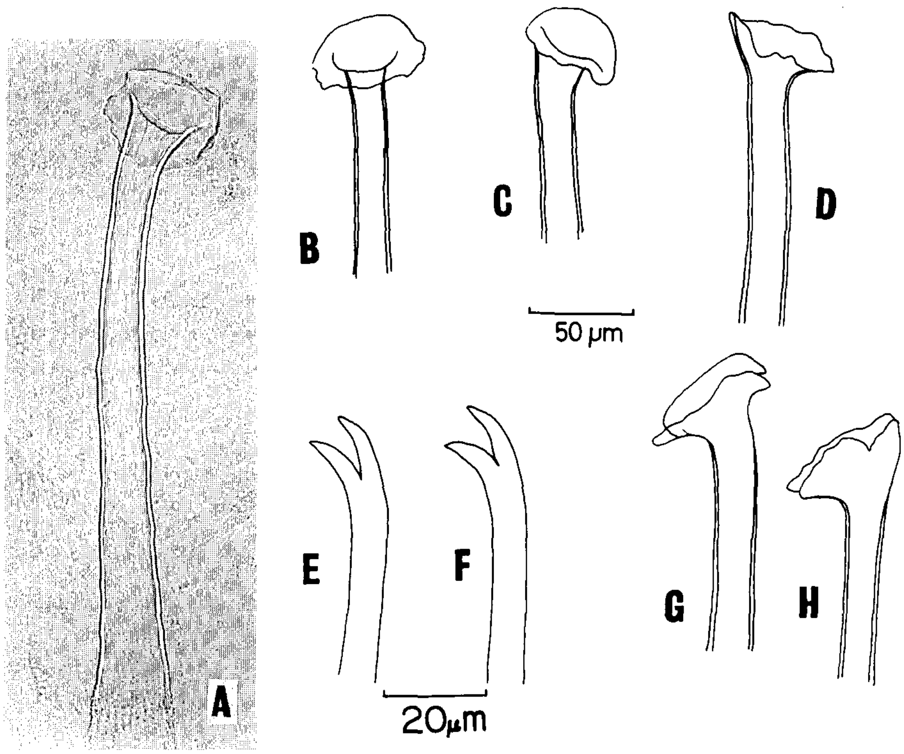

Fig. 4. Penis sheaths and chaetae of "plate-topped" form (A-F) and intermediate types of penis sheaths between "typical" and "plate-topped" types (G, H) of Japanese *L. hoffmeisteri*. A, B, normal "plate-topped" type of penis sheaths (R. Shinkawa, Sapporo); C, D, trumpet-shaped sheaths (C, L. Suwa; D, Osaka); E, F, chaetae (L. Suwa). G, H, intermediate types of sheaths (R. Shinkawa, Sapporo). All figures except A show only distal ends.

Results

1. Comparison of penial and chaetal characters

1.1. Penis sheath

Three additional types of penis sheaths that were somewhat different from "typical" and "plate-topped" types were occasionally found in the present study. One is the penis sheath having a clumped distal end (Fig. 3C, D), that was found in specimens from some lakes, e.g., Lake Shikotsu, L. Kuttara and L. Ō-numa. We regarded this type of penis sheaths as "typical" type deformed by copulation because: 1) this type was restricted in mated specimens; 2) when this type was found only in one of the paired sheaths, the other sheath could be always assigned to "typical" type (Fig. 3, compare E with F); and 3) all the rest individuals simultaneously collected with the individuals having clumped-ended penis sheaths had typical "typical" type penis sheaths.

The second type is the penis sheath with a trumpet-shaped end on a straight shaft (Fig. 4C, D), which has been frequently found in many localities. Ohtaka (1985) regarded this type as intermediate between "typical" and "plate-topped" types. In the present study, however, we included this type in "plate-topped" type, because its straight shaft and distal end resemble those of typical "plate-

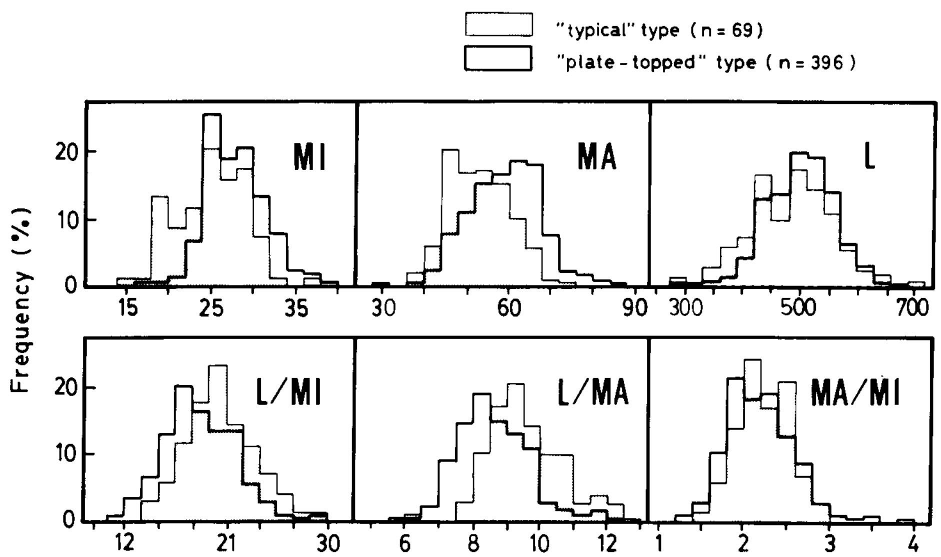

Fig. 5. Frequency distribution of minimum diameter (MI), maximum diameter (MA) and length (L) of penis sheaths and their ratios in the two forms of Japanese *L. hoffmeisteri*. All specimens measured of these two forms are combined.

topped" type more closely than those of "typical" type.

The third type is the penis sheath that is intermediate in shape between "typical" and "plate-topped" types (Fig. 4G, H). Interestingly, this type was found only in localities where both "typical" and "plate-topped" types occurred (cf. later).

Excluding these atypical penis sheaths, "typical" and "plate-topped" types of penis sheaths were compared (Table 1). Each value of the measurements of penis sheaths is a little larger, and each ratio is a little smaller in "plate-topped" type than in "typical" type (Table 1; Fig. 5). "Plate-topped" type has proportionally smaller L and larger MI and MA values than "typical" type (Fig. 6), then appears thicker than the latter. Average values of the MA and all ratios are significantly different between "typical" and "plate-topped" types (t-test: $0.001 > P$ in MA, L/MI and L/MA; $0.05 > P > 0.01$ in MA/MI).

1.2. Chaetal teeth

The UT/LT ratio was approximately 1 on the average, and there was no significant difference between "typical" and "plate-topped" forms (Table 1; Fig.

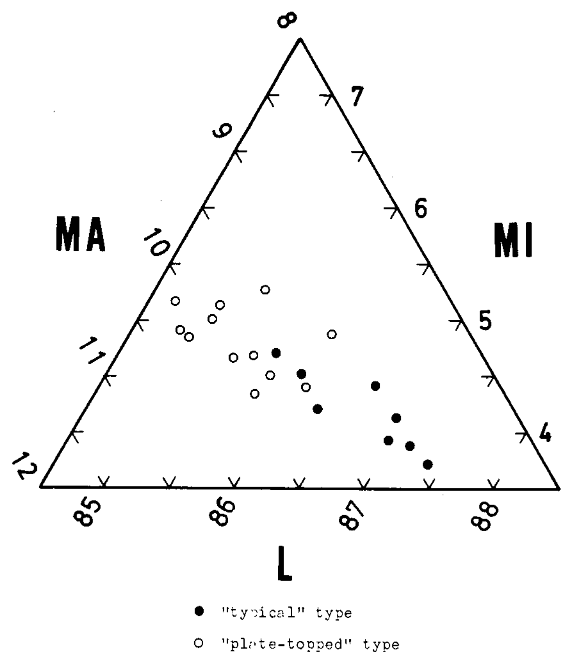

Fig. 6. Triangular diagram of the length (L), minimum diameter (MI) and maximum diameter (MA) of penis sheaths in the two forms of Japanese *L. hoffmeisteri*. Open circles, "plate-topped" type; solid circles, "typical" type. Each circle represents the average value of each local sample.

Table 1. Measurements and ratios of penis sheaths and chaetae in the "typical" form and "plate-topped" form of Japanese *L. hoffmeisteri*. Specimens from 8 localities were used for the "typical" form, and those from 12 localities for the "plate-topped" form.

	"Typical" form Mean±SD (range)	"Plate-topped" form Mean±SD (range)
Penis sheath		
No. of specimens examined	69	396
MI (μm)	24.3±4.2 (15-36)	27.3±3.9 (16-58)
MA (μm)	52.1±7.8 (36-75)	57.8±9.1 (30-90)
L (μm)	490.6±81.3 (280-704)	498.9±62.0 (284-689)
L/MI	20.5±3.1 (13.5-29.1)	18.6±3.1 (11.1-31.9)
L/MA	9.5±1.1 (7.6-12.2)	8.7±1.3 (5.9-13.2)
MA/MI	2.2±0.2 (1.5-2.9)	2.1±0.4 (1.9-3.9)
Chaeta		
No. of specimens examined	145	183
UT/LT	1.05±0.16 (0.58-1.72)	1.02±0.10 (0.81-1.27)

3 G, H; Fig. 4E, F). Considerable variations were found within and among localities, in particular in "typical" form; the largest ratios (UT/LT$>$1.5) were found in worms from Lake Okotanpe and L. Shikotsu in Hokkaido (Fig. 3I), and the smallest ratio (UT/LT=0.58) from the southwestern shore in Lake Ō-numa, Hokkaido (Fig. 3J).

2. Distribution

Both "typical" and "plate-topped" forms were widely distributed in diverse habitats in Japan, including lentic and lotic waters (Fig. 7). Their distribution ranges were greatly overlapped. Out of a total of 55 localities surveyed, only the "typical" form was collected from 30 localities, only the "plate-topped" form from 15, and both forms from ten. Individuals having the intermediate type of penis sheaths were collected from four out of ten localites where the two forms were syntopic.

The "typical" form is mainly found in oligotrophic less polluted waters (Table 2), though they occasionally found from heavily polluted ones in urban areas. On the other hand, "plate-topped" form was found in eutrophic or organically polluted waters, and was rare in oligotrophic lakes whose modified Carlson's trophic state index (Aizaki *et al.*, 1981) was less than 50 (Table 2). Co-occurrence of the two forms were often found where the polluted and non-polluted waters met.

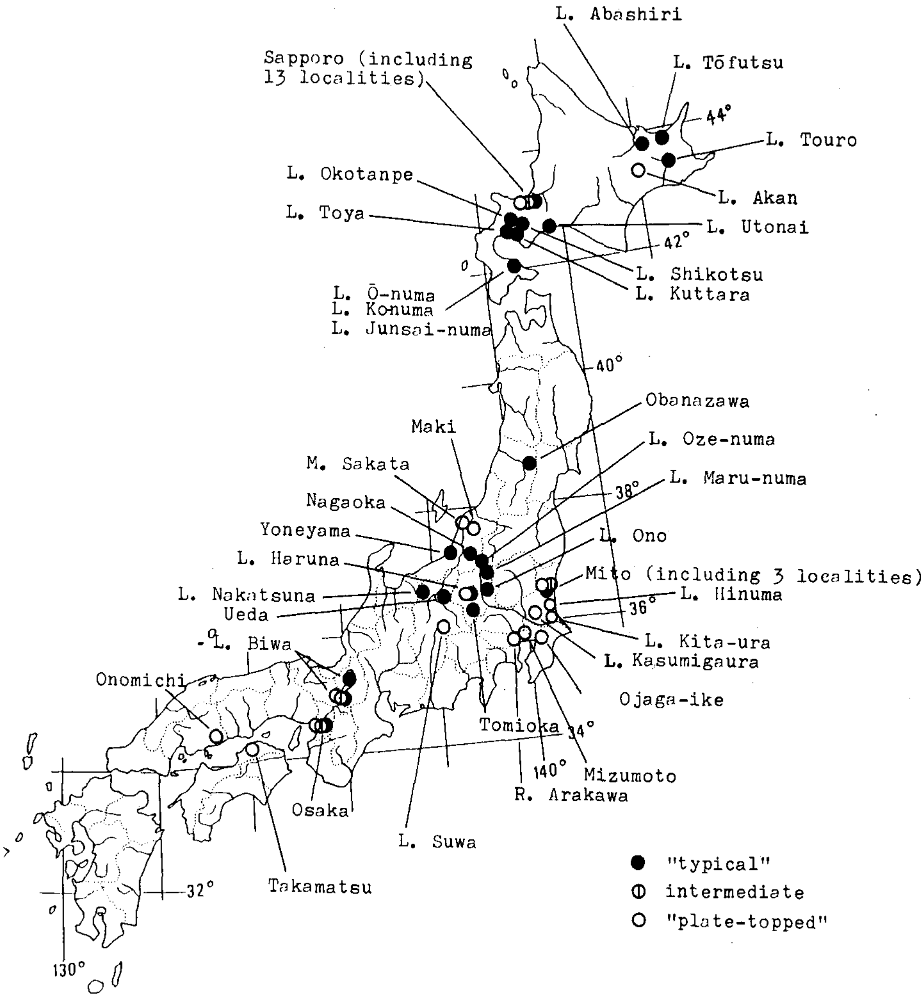

Fig. 7. Records of the two forms of *L. hoffmeisteri* and their intermediates in Japan.

Concluding Remarks

The present study detected the following facts: 1) There is no reliable character discriminating between the two forms other than the shape of penis sheath. 2) Most Japanese specimens can be assigned to either "typical" or "plate-topped" form in accordance with morphological criteria of penis sheaths,

Table 2. A list of *L. hoffmeisteri* dwelling lakes of which modified Carlson's trophic state indexes have been estimated. Numerals in parentheses indicate the index values calculated from Secchi disk transparency (Aizaki *et al.*, 1981).

"Typical" form

L. Shikotsu (12*); L. Kuttara (24); L. Toya (28); L. Okotanpe (30); L. Maru-numa (33**); L. Biwa, North basin (37*); L. Nakatsuna (40*); L. Oze-numa (41**); L. Ō-numa (43*); L. Haruna (43*); L. Ono, Mt. Akagi (46**); L. Biwa, South basin (52**)

"Plate-toppped" form

L. Haruna (43*); L. Biwa, South basin (52*); L. Kita-ura (56); L. Kasumigaura (66*); L. Hinuma (72*); L. Suwa (77*)

Sources of index values: * Aizaki *et al.* (1981); ** Yajima (pers. comm.); others, Ohtaka (unpubl.).

though worms with intermediate conditions of penis sheaths were rarely found where two forms occurred syntopically. 3) Both "typical" and "plate-topped" forms were widespread in Japan, and occurred syntopically in ten out of 55 surveyed localities. 4) There is habitat difference between them, that is, "typical" form mainly occurred in oligotrophic or non-polluted waters whereas "plate-topped" form in eutrophic or organically polluted waters.

From these facts, it is certain that the Japanese "typical" and "plate-topped" forms of *Limnodrilus hoffmeisteri* are not subspecies of a single species. Their distribution ranges widely overlapped, and furthermore, both forms were collected from ten out of 55 surveyed localities. However, taxonomic status of these two forms are yet not clear. Two interpretations still remain; They are 1) different species or 2) mere infraspecific variations.

Unfortunately, all the facts enumerated above are equally consistent with these two alternative hypotheses. Under such circumstances, we consider that it is better to keep the Japanese two forms as ecomorphs of a single species to avoid taxonomic confusion.

Acknowledgements

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Appendix. A list of materials examined

Numbers of mature (M) and immature (I) specimens used, including material examined and listed in my previous paper, are given. The mature stage was defined as the stage having some or all genital organs in addition to gonads.

"Typical" form: 3M, Lake Tōfutsu, Hokkaido (43°56′N, 144°26′E; western shore, sandy mud): 14 Aug. 1982. 4M, Lake Abashiri, Hokkaido (43°56′N, 144°10′E; littoral zone off Memanbetsu, mud with plant debris): 10 May 1981; 13 Aug. 1982. 9M, Lake Touro. Hokkaido (43°09′N, 144°30′E: western shore, mud with plant debris): 5 May 1981; 17 Aug. 1982. 10M, Lake Barato, Ishikari, Hokkaido (43°11′N, 141°20′E; western basin with 3-9 m in depth, mud with plant debris): 9 June 1982; 6 June, 8 Aug., 12 Oct. 1983. 2M, Pond Pekeretto, Sapporo, Hokkaido (43°10′N, 141°23′E; shore, mud with large amount of plant debris): 11 May 1983.

5M, River Fushiko, Sapporo, Hokkaido (43°09′N, 141°22′E; mud with large amount of plant debris): 11 May 1983. 3M, Pond Moere, Sapporo, Hokkaido (43°08′N, 141°26′E; littoral, mud with large amount of plant debris): 6 July 1982. 3M, River Shinoroshinkawa, Sapporo, Hokkaido (43°07′N, 141°25′E; sandy mud): 6 May 1983. 2M, River Sousei, Sapporo, Hokkaido (43°07′N, 141°20′E; organically polluted river, mud with plant debris): 9 June 1982. 11M, River Shin-kawa, Sapporo, Hokkaido (43°06′N, 141°20′E; junction with River Kotoni, mud with plant debris): 6 July 1982. 17M, Sakusyu-kotonigawa brook, Sapporo, Hokkaido (43°05′N, 141°20′E; small current on the campus of Hokkaido University, mud with large amount of plant debris): 16 Apr., 1, 16 May 1983. 7M, Botanical Garden, Hokkaido University, Sapporo, Hokkaido (43°03′N, 141°21′E; pond, mud): 23 May 1983. 10M, Maruyama, Sapporo, Hokkaido (43°03′N, 141°19′E; a brook near Maruyama Zoo, mud with large amount of plant debris): 2 Dec. 1982; 11 Apr., 2 May, 30 Dec. 1983. 2M, River Tsukisamu-raunenaigawa, Sapporo, Hokkaido (43°01′N, 141°21′E; near Hokkaido Agricultural Experiment Station, mud): 15 June, 1982. 5M, Nishioka reservoir, Sapporo, Hokkaido (42°59′N, 141°23′E; southern shore, mud with large amount of plant debris): 1 Sep. 1982; 26 Apr. 1983. 3M, River Toyohira, Sapporo, Hokkaido (42°55′N, 141°09′E; middle course of the river near Jozankei Spa, mud with leaf litter): 10 Nov. 1984; R.B. Kuranishi collected. 12M, Lake Okotanpe, Hokkaido (42°48′N, 141°16′E; profundal zone with 14-22 m in depth, mud): 8 Aug. 1984. 8M+2I, Lake Shikotsu, Hokkaido (42°46′N, 141°24′E; littoral zone off Kohan, 20-28 m deep, mud): 28 Apr. 1984. 1M, Lake Utonai, Hokkaido (42°42′N, 141°43′E; littoral, gravely mud): 22 June, 1982. 1M, Lake Toya, Hokkaido (42°36′N, 140°48′E; littoral zone off the Toya Hydrobiological Station, Hokkaido University, 17-22 m deep, sandy mud): 30 May 1983. 14M, Lake Kuttara, Hokkaido (42°30′N, 141°11′E; littoral zone, 5-15m deep, mud): 10 July 1983. 1M, Lake Junsai-numa, Hokkaido (42°00′N, 140°38′E; northern shore, mud with plant debris): 14 July 1982. 3M, Lake Ko-numa, Oshima, Hokkaido (41°59′N, 140°40′E; northern shore, mud with large amount of plant debris): 18 May 1982. 14M+6I, Lake Ō-numa, Oshima, Hokkaido (41°59′N, 140°41′E; southwestern shore and littoral region, 0-7 m deep, mud): 18 May, 14 July 1982; 29 May 1984. 65M, Obanazawa, Yamagata prefecture (38°37′N, 140°24′E; paddy field, mud with large amount of plant debris): 13 May 1981. 1M, Naganeyama hill, Obanazawa, Yamagata prefecture (38°36′N, 140°26′E; brook, mud with large amount of plant debris): 29 Aug. 1984. 12M, Nagaoka, Niigata prefecture (37°26′N, 138°52′E; paddy field near the Nagaoka Branch, Niigata University, mud with plant debris): 13 June 1979. 10M, Yoneyama, Niigata prefecture (37°18′N, 138°27′E; paddy field, mud): 19 Apr. 1979. 5M, Lake Oze-numa, Gunma prefecture (36°56′N, 139°18′E; profundal zone, 6-9 m deep, mud), 23 Jul. 1986. 2M, Lake Maru-numa, Gunma prefecture (36°49′N, 139°21′E; profundal): 1 July 1980; staff of the National Institute for Environmental Studies collected. 1M, Lake Ono, Gunma prefecture (36°33′N, 139°11′E; 1345 m alt. of Mt. Akagi, profundal zone with 10 m in depth, mud): 9 Nov. 1980. 4M, Lake Haruna, Gunma prefecture (36°28′N, 138°52′E; 1084 m alt. of Mt. Haruna, profundal zone with 12 m in depth, mud): 14 June 1981. 2M, River Takada, Tomioka, Gunma prefecture (36°15′N, 138°52′E; mud with plant debris): 4 Apr. 1984. 10M, Mito, Ibaraki prefecture (36°24′N, 140°27′E; a brook in paddy field, mud with plant debris): 21, 27, 28 Apr. 1981. 4M, Pond Senba-ko, Mito, Ibaraki prefecture (36°22′N, 140°28′E; western shore, mud with plant debris): 28 Oct. 1980; 8 Apr., 6 July 1981. 2M, Ueda, Nagano prefecture (exact locality and sampling date unknown): T. Sugimoto collected. 2M, Lake Nakatsuna, Nagano prefecture (exact locality unknown, 7.9 m deep): 11 Nov. 1984; K. Hirabayashi collected. 4M, Lake Biwa (north basin), Shiga prefecture (exact locality unknown, sublittoral zone off Wani, sandy mud): 7 Aug. 1981. 5M, Lake Biwa (south basin), Shiga prefecture (35°04′N, 135°54′E; center of the basin with 5 m in depth, mud with plant debris): 7 Aug. 1981. 10M, Osaka, Osaka prefecture (34°37′N, 132°32′E; a small pond in Nagai Park, mud): 13 Oct. 1982.

"Plate-topped" form: 28M, Lake Akan, Hokkaido (43°26′N, 144°05′E; off Akan Spa, 10-12 m deep, mud): 15 Aug. 1982. 5M, Lake Barato, Ishikari, Hokkaido (43°11′N, 141°20′E; western basin with 3-9 m in depth, mud with plant debris): 9 June 1982; 6 June, 8 Aug., 12 Oct. 1983. 2M, River Sousei, Sapporo, Hokkaido (43°07′N, 141°20′E; organically polluted river, mud with plant debris): 9 June 1982. 8M, River Shinkawa, Sapporo, Hokkaido (43°06′N, 141°20′E; junction with River Kotoni, mud with plant debris): 6 July 1982. 9M, Maruyama, Sapporo, Hokkaido (43°03′N, 141°19′E; a brook near Maruyama Zoo, mud with large amount of plant debris): 2 Dec. 1982; 11 Apr., 2 May, 30 Dec. 1983. 2M, River Tsukisamu-raunenaigawa, Sapporo, Hokkaido (43°01′N, 141°21′E; near Hokkaido Agricultural Experiment Station, mud): 15 June 1982. 7M, Lake Hyo-ko, Suibara, Niigata prefecture (37°50′N, 139°14′E; southern shore, mud with plant debris): 24 Sep. 1981; K. Hayashi collected. 31M, Marsh Sakata, Maki, Niigata prefecture (37°49′N, 138°53′E; mud with large amount of plant debris): 5 Apr., 16 June, 24 July, 5 Dec. 1981; K. Hayashi and Y. Abe collected. 4M, Nikatsutsumi reservoir, Maki, Niigata prefecture (37°47′N, 138°52′E; shore, mud with plant debris): 7 Oct. 1982. 1M, Maki, Niigata prefecture (37°45′N, 138°53′E; paddy field, mud with large amount of plant debris): 7 Oct. 1982. 2M, Lake Haruna, Gunma prefecture (36°28′N, 138°52′E; 1084m alt. of Mt. Haruna, profundal zone with 12 m in depth, mud): 14 June 1981. 100 M, Mito, Ibaraki prefecture (36°24′N, 140°27′E; a brook in paddy field, mud with plant debris): 21, 27, 28 Apr. 1981. 10M, Pond Senba-ko, Mito, Ibaraki prefecture (36°22′N, 140°28′E; western shore, mud with plant debris): 28 Oct. 1980; 8 Apr., 6 July 1981. 2M, Lake Hinuma, Ibaraki prefecture (36°17′N, 140°27′E; western part of the lake, mud): 9 Aug. 1982; H. Morino collected. 2M, Lake Kasumigaura, Ibaraki prefecture (36°05′N, 140°24′E; mud): 9 Mar. 1982; staff of the National Institute for Environmental Studies collected. 225M+over 501, Lake Kita-ura, Ibaraki prefecture (36°01′N, 140°34′E; center of the lake with 6m in depth, mud): monthly sampling carried out from Apr. 1980 to Feb. 1982. 5M, Mizumoto, Tokyo prefecture (35°46′N, 139°53′E; ponds in the Tokyo Fisheries Experimental Station, mud with plant debris): 6, 29 July 1983; K. Hayashi collected. 37M+261, River Arakawa, Tokyo prefecture (35°43′N, 139°50′E; organically polluted estuary near Kinegawa Bridge, mud): 5 July 1981. 1M, Ojaga-ike reservoir, Chiba prefecture (35°34′N, 140°20′E; shore, mud with plant debris): 30 May 1981. 67M, Lake Suwa, Nagano prefecture (36°03′N, 138°05′E; center of the lake with 6m in depth): 17 Oct., 3 Nov. 1980; 20 Aug., 30 Nov. 1981. 5M, Lake Biwa (south basin), Shiga prefecture (35°04′N, 135°54′E; center of the basin with 5 m in depth, mud with plant debris): 7 Aug. 1981. 20M, Akashi, Hyogo prefecture (34°39′N, 135°00′E; a pond in Akashi Park, mud with plant debris): 8 Oct. 1982. 20M, Osaka, Osaka prefecture (34°37′N, 132°32′E; a small pond in Nagai Park, mud): 13 Oct. 1982. 26M, Onomichi, Hiroshima prefecture (34°28′N, 133°19′E; a current at Bingo-akasaka, mud): 9 Oct. 1982. 6M, River Gobou, Takamatsu, Kagawa prefecture (34°18′N, 134°01′E; organically polluted river, mud with plant debris): 11 Oct. 1982.