Zoologica Scripta, Vol. 20, No. 4, pp. 413–417, 1991
Printed in Great Britain

0300–3256/91 $3.00 + .00
Pergamon Press plc

Lappodiamesa willasseni sp.n. (Diptera, Chironomidae, Diamesinae) from the Soviet Far East

EVGENIJ A. MAKARCHENKO and IRINA E. KERKIS

Accepted 13 *May* 1991

Makarchenko, E. A. and Kerkis, I. E. 1991. *Lappodiamesa willasseni* sp.n. (Diptera, Chironomidae, Diamesinae) from the Soviet Far East.—*Zool. Scr. 20:* 413–417.

Lappodiamesa willasseni sp.n. is described. It differs from related species in characteristics of the male adult, the pupa and the larva. A key to the species of *Lappodiamesa* Serra-Tosio is given. In contrast to previously described *Diamesinae*, the homologes of the large polytene chromosomes are often unpaired in few regions and they have a meandric structure. The four pairs of chromosomes include three metacentric and one acrocentric (spherical) chromosome. A series of chromosome markers are described.

Evgenij A. Makarchenko, Academy of Sciences of the U.S.S.R., Far East Branch, Institute of Biology and Soil, 690022 Vladivostok-22, U.S.S.R.
Irina E. Kerkis, Academy of Sciences of the U.S.S.R., Siberian Branch, Institute of Cytology and Genetics, 630090 Novosibirsk-90, U.S.S.R.

Introduction

Based on two male imagines from northern Sweden, Serra-Tosio (1968) described the monotypic genus *Lappodiamesa* Serra-Tosio and its type species *Lappodiamesa brundini* Serra-Tosio. More recently, Makarchenko (1983, 1985) described the larvae and pupae of this species from Chukotka Peninsula. He indicated the similarity between *L. brundini* and *Diamesa (Syndiamesa) vidua* Kieffer, known from Novaya Zemlya (Kieffer 1922). However, not having access to the type material, Makarchenko was reluctant to formally synonymize the two species. Later, Saether & Willassen (1988) studied the lectotype of *D. vidua* and concluded that *Lappodiamesa vidua* (Kieffer) is the correct name of the species. In the same paper, they also described from North America a second species of the genus, *Lappodiamesa boltoni* Saether & Willassen. In South Primorye (Soviet Far East) a third species has recently been discovered. It will be described below and a key to male adults, to pupae and to larvae of the genus is given.

It is well known that the polytene chromosomes of Chironomidae have a distinct banding pattern useful as characters in taxonomic and phylogenetic studies (Bauer 1936; Martin 1979), especially for the genus *Chironomus* Meigen. To our knowledge, only about 10 karyotypes have been described from the subfamily *Diamesinae*. These karyotypes represent three genera and do not include *Lappodiamesa*. In order to broaden our understanding of karyotype variation within the Chironomidae we also studied the karyological characteristics of *Lappodiamesia willasseni* sp.n., a species described in this paper.

Material and methods

Specimens of *L. willasseni* were fixed in either Udemans solution (adults) or 70% ethanol (larvae and pupae), treated in 10% KOH solution for maceration of soft tissues, and finally mounted for microscopy in Euparal.

A total number of 22 larvae for karyological analysis were fixed in absolute ethanol-acetic acid (3:1) and stored in a refrigerator. Analysis of polytene chromosomes was performed on squashed salivary glands prepared by the standard aceto-orcein technique. Banding patterns were analyzed in the external zone of the glands, where the level of polyteny is highest and the chromosomes are of large size.

The gonads and the imaginal discs, stained with aceto-orcein, were used for the meiotic and metaphase chromosome spreads. Determination of larval age was based on the shape of the imaginal discs according to Wülker & Götz (1968).

Abbreviations and terminology in the description of the adult, pupa and larva follow Saether (1980).

Key to species of *Lappodiamesa* Serra-Tosio

Males

1. Acrostichals present. AR=1.4–1.7 . . . . . *L. vidua* (Kieffer). Palaearctic.
— Acrostichals absent. AR=2.3–2.6 2
2. Anal point 53–105 μm long, with apical hair sensillum or more stout with double to triple apical spines . . . . . *L. boltoni* Saether & Willassen. Nearctic.
3. Anal point 112–115 μm long, simple and without hair sensillum . . . . . *L. willasseni* sp.n. Palaearctic.

Pupae

1. Anal lobe with apical tubercle or spine 2
— Anal lobe without apical tubercle or spine . . . . . *L. boltoni* Saether & Willassen

2. Lateral setae on all abdominal segments with 3 or more branches . . . . . *L. willasseni* sp.n.
– Lateral setae simple or sometimes with 2 branches *L. vidua* (Kieffer)

Fourth instar larvae

1. Premandible with 3 teeth, procercus with 7 anal setae . . . . . *L. willasseni sp.n.*
– More than 3 teeth on premandible, procercus with 8–9 setae 2
2. Premandible with 4 teeth, middle tooth of mentum equally high and wide as first lateral tooth . . . . . *L. vidua* (Kieffer)
– Premandible with 5 teeth, middle tooth of mentum considerably wider than first lateral tooth . . . . . *L. boltoni* Saether & Willassen

***Lappodiamesa willasseni* sp.n** (Figs 1–3)

Type material. Holotype ♂, from Soviet Far East, Primorye, Khasan Region, Narva River, 25 March 1989, L. Kolomyc leg. Paratypes, 2 ♂♂, 2 pupae, 18 larvae, same data as holotype except: 25 March–14 April 1989, L. Kolomyc and E. Makarchenko leg.; 26 larvae: same data as holotype except: 7 March 1989 and 2 March 1989, E. Makarchenko leg. Deposited in the Academy of Sciences of the U.S.S.R., Far East Branch, Institute of Biology and Soil, Vladivostok.

Diagnosis. Antennal ratio of male adult 2.3–2.6, acrostichals absent, anal point 112–115 μm. All lateral setae of pupal abdomen branched, anal lobe with distinct apical tubercle. Premandible of larva with 3 teeth, median tooth of mentum 4–4.5 times wider than the first lateral tooth, procercus with 7 long anal setae.

Description of morphology

Male imago (n=2). Total length 4.0–4.6 mm, ratio length/wing length 1.1–1.3, grayish-brown.

Head. AR 2.3–2.6. Head with 4–5 inner vertical setae (42.5–55 μm long), 4 outer verticals (10–12.5 μm), 16–17 postorbitals (30–35 μm), and 7–12 setae (100–142.5 μm) on clypeus. Eyes pubescent between all ommatids.

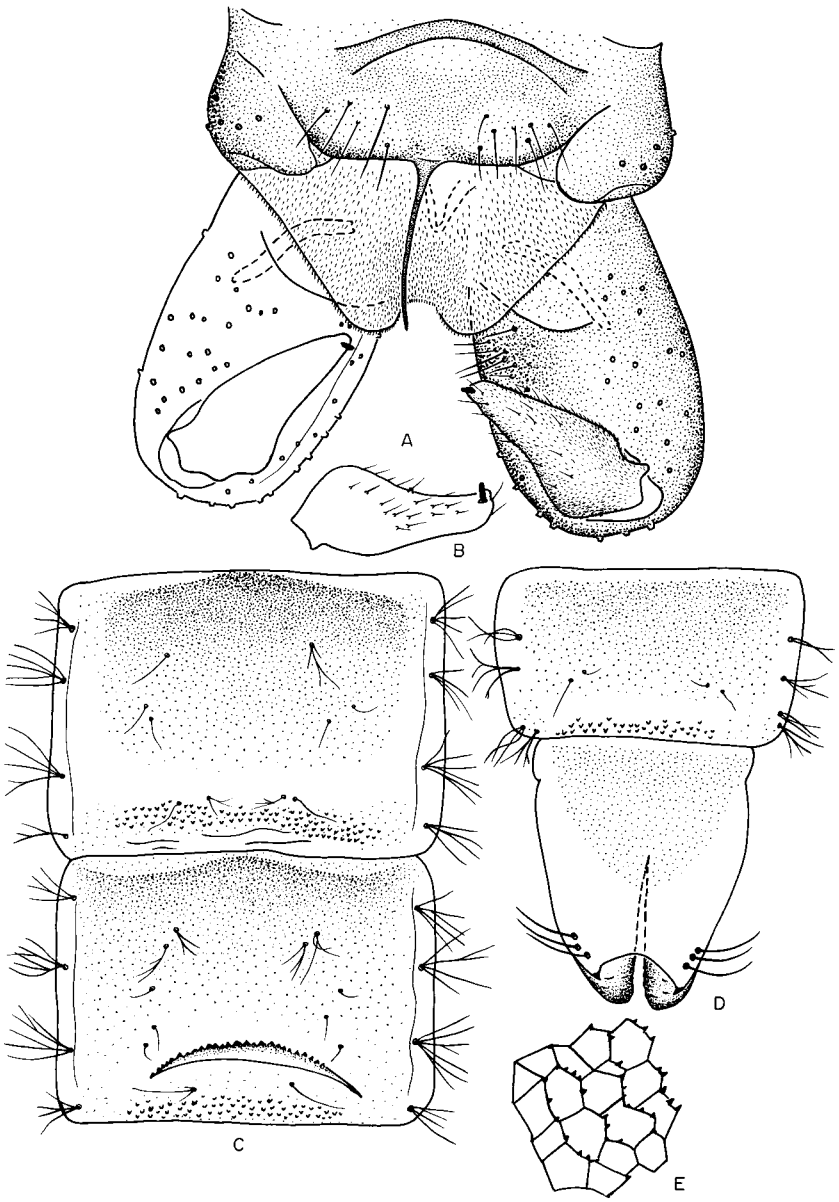

Fig. 1. Lappodiamesa willasseni sp.n.—*A–B.* Male.—*C–E.* Pupa.—*A.* Hypopygium.—*B.* Gonostylus.—*C.* Tergites V–VI.—*D.* Tergites VIII–IX.—*E.* Surface of tergites.

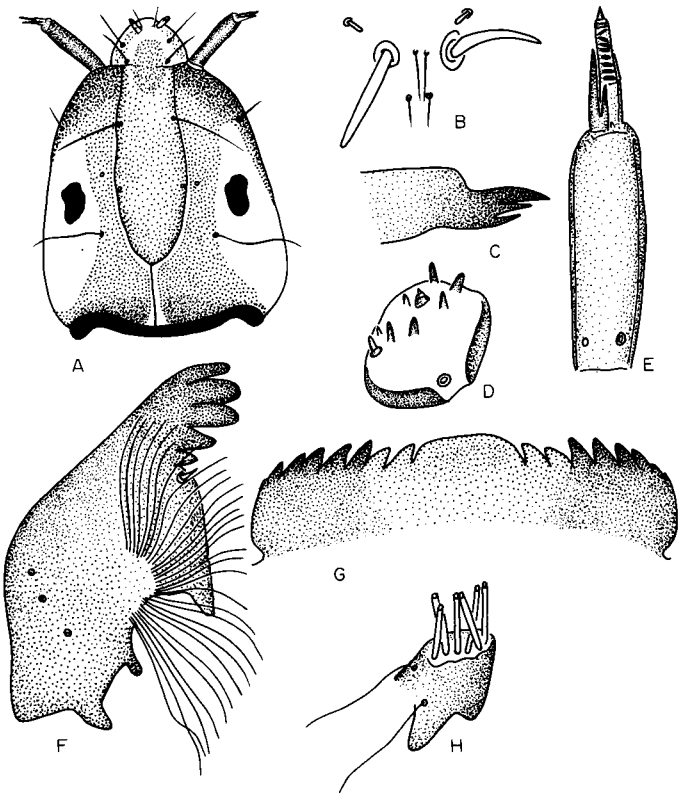

Fig. 2. Lappodiamesa willasseni sp.n., larva. — *A*. Head. — *B*. SI–SIV sensilla of labrum. — *C*. Premandible. — *D*. Maxillary palp. — *E*. Antenna. — *F*. Mandible. — *G*. Mentum. — *H*. Procercus.

Length of four ultimate palp segments (μm): 135, 205, 163, 253. Ratio head width/palp length 1.37.

Thorax. Antepronotum with 6–7 lateral setae (75–88 μm long). Acrostichals and supraalars absent; dorsocentrals 4–6, prealars 12–16, scutellars 29–34.

Wing. Wing length 3.5 mm. Microtrichia of wing membrane visible under 100 x magnification. Squama with 65–70 setae (93–110 μm). R and R_1 with 13 setae, R_{4+5} with 3. Anal lobe with well-developed projection.

Legs. Spur of front tibia 80 μm long, spurs of middle tibia 58–69 μm, of hind tibia 55–75 μm. Apex of hind tibia without distinct setal comb. Pulvilli as small spines. Lengths and proportions of legs (μm):

	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5	LR	SV	BV	BR
P_1	1069	1269	802	451	284	175	134	0.63	2.92	3.0	6.8
P_2	1119	1162	534	326	217	134	134	0.46	4.25	3.5	4.8
P_3	1420	1420	735	434	251	150	134	0.52	3.86	3.7	9.3

Hypopygium (Fig. 1A–B). Tergite IX narrow and with 5–8 setae on each side. Laterosternite with 5–8 setae, 120–155 μm long. Anal point dark brown, thin and long (113–115 μm), without apical hair sensillum. Gonocoxite 257–271 μm long; basally with rounded superior volsella, inferior volsellae weakly delineated. Gonostylus 141–149 μm long, wide at basal part and narrow towards apex; megaseta 14–16 μm long.

Pupa ($n = 2$). Total length 3.6–4.2 mm. Exuviae yellow-gray, cephalothorax dark brown, abdomen grayish-brownish.

Cephalothorax. Frontal warts, cephalic tubercles and frontal setae absent. Thorax strongly rugulose without thoracic horn. Median antepronotals 53 and 66 μm long, lateral antepronotal setae 62 μm long.

Abdomen (Fig. 1C–E). Surface of tergites and sternites with anteriomedian, net-like shagreen; posterior shagreen of tergites II–VIII composed of 2–4 rows of spinules; sternites VI–VIII with weak shagreen and posteriorly with about 20 short spines in transverse row. Dorsal setae mostly with two or more branches. Segment I with 1 L-seta inserted in posterolateral corner and composed of 4–5 branches; segments II–VIII with 4 L-setae, each with 6–10 branches. Anal lobe with dark, acute, triangular spine. Anal macrosetae 100–110 μm long. Male genetal sack slightly longer than anal lobe.

Fourth instar larva (Fig. 2A–H)($n = 12$). Total length 5.2–7 mm.

Head. Head capsule 0.28 mm long, 0.25 mm wide; yellow-brown, frontoclypeal apotome brown, submentum light brown, genae yellow around eye spots. Antenna (Fig. 2E) light brown to brown; length of antennal segments 1–5 (μm): 80, 15, 14, 3, 4; AR 2–2.3; large and small ring organs situated near base of first segment, antennal blade 30 μm long and ending near base of fourth segment. Labrum with simple S_I–S_{IV} setae (Fig. 2B). Premandible with 3 teeth (Fig. 2C). Mandible (Fig. 2F) dark brown, apical tooth narrower than lateral teeth; seta interna with 30–34 branches, seta subdentalis short and peg-like. Maxillary palp (Fig. 2D) with 9–10 peg- or cone-like sensilla. Anterior margin of mentum (Fig. 2G) inconspicuously convex, with one median and 7–8 lateral teeth,

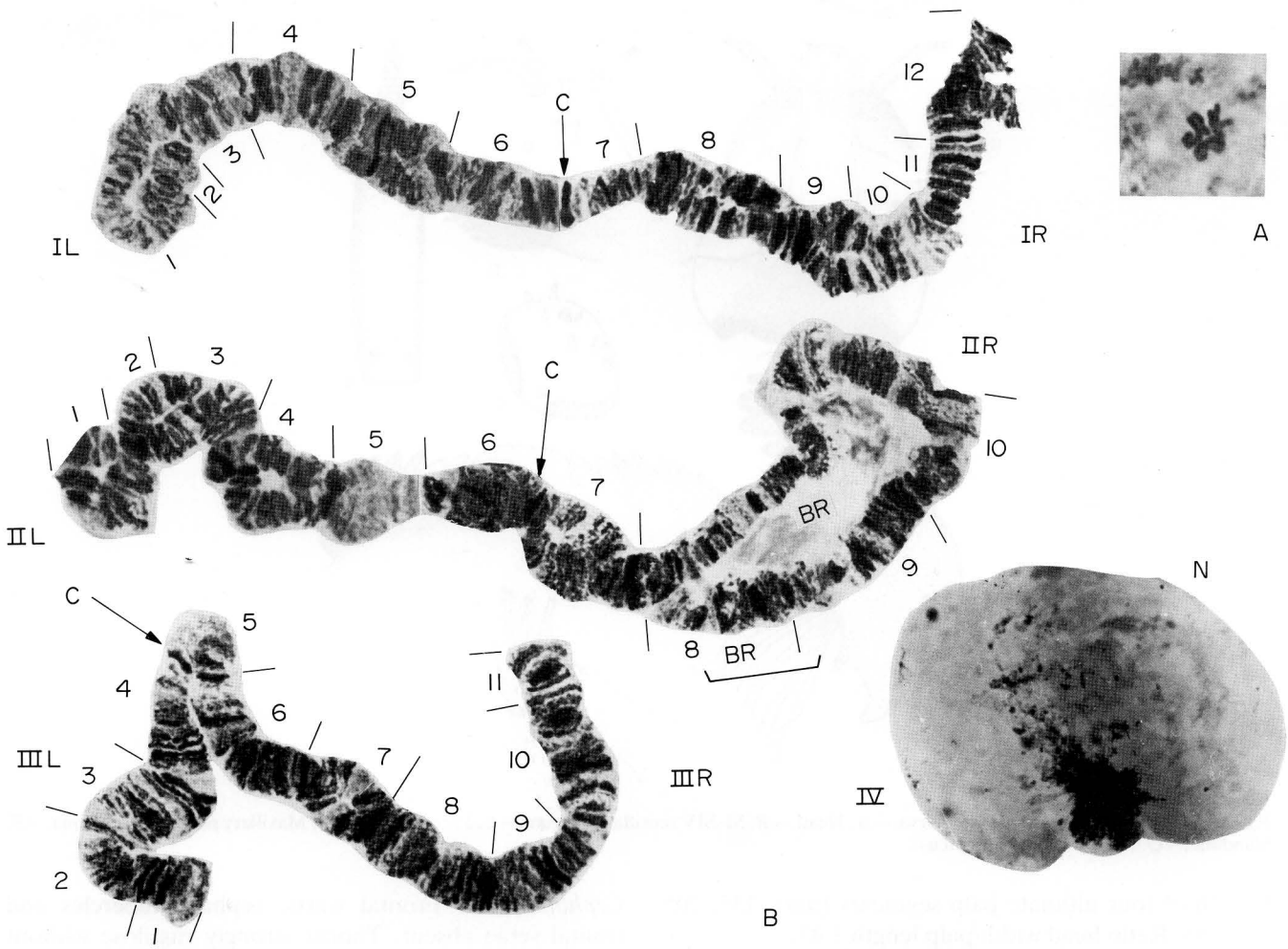

Fig. 3. Lappodiamesa willasseni sp.n., entire chromosome complement.—*A*. Mitotic chromosomes.—*B*. Standard photomap of the salivary gland polytene chromosomes, IL–IR, IIL–IIR, IIIL–IIIR arms of chromosomes I–III, respectively; IV spherical chromosome; N nucleolar organizer; BR Balbiani ring. Arrows indicate putative candidates for centromeric bands.

median tooth 4–5 times broader than first lateral tooth; first and second lateral teeth slightly lower than third and lighter than remaining lateral teeth.

Abdomen. Procercus (Fig. 2H) dark brown, about as wide as long, with 7 anal setae and 2 subapical setae.

Karyological description of fourth instar larvae

Salivary glands. Salivary glands sack-shaped, internal cavity filled with secretion. Cells more or less polygonal, total numbers per gland 104–125. Cells of internal zone (Kuberskaya 1984) conical, containing fine granular secretion; cells of external zone polygonal. Cytoplasm with large granules.

Chromosomes. Four pair present (Fig. 3A, mitotic metaphase of imaginal disc); two pairs metacentric, one pair submetacentric, one acrocentric.

Polytene salivary gland chromosomes (Fig. 3B)
Chromosome I metacentric, 215 ± 4 μm long, containing sections 1–12; easily recognised by absence of homolog pairing in sections 1–2 and the fan-like telomeric region 12. The centromeric region is identified in section 6/7. Good chromosome markers may be a band reminiscent of an open circle in section 3/4 and the light swelling in section 5 and 10.

Chromosome II metacentric, 210 ±7 μm, with centromeric region in section 6/7. Markers are a constriction and a distinct band in section 4/5, a light swelling in section 5, a constriction in region 7, and a clear-cut band in telomeric region 10. A small inversion involving the site of the Balbiani ring is identified in section 8.

Chromosome III metacentric, 187 ± 2 μm long. The following markers may be identified: a block of closely adjacent bands in section 2, a pair of dark bands in section 9, a light swelling in section 6 where the homologs of the polytene chromosome often do not pair; precentromeric region 4 containing three light bands, of which one is centromeric; section 2 with light interval, and three light internal bands in section 1.

Chromosome IV acrocentric, spherical with heterochromatin block in the centre. Heterochromatin block of loose structure, with projecting fibers devoid of banding patterns.

Remarks

Most of the chromosomes were taken from the salivary gland cells of prepupae. The chromosomes had a meandric structure and the banding patterns were indistinct.

Chromosome IV does not, as a rule, associate to the chromosomes in the set, and it occupies a chance position in the nucleus. Similar spheric chromosomes have been described from other representatives of the subfamily

Diamesinae, including *Pseudodiamesa nivosa* (Goetghebuer) and *Pseudodiamesa branickii* (Nowicki) (Bauer 1936; Kuberskaya 1974, 1979; Petrova 1983, 1986; Zacharias 1984). Thus, it may be concluded that *L. willasseni* has features in common with other studied species of the subfamily. Other similarities concern the structure, shape and size, as well as the fine granular appearance of the salivary gland cells (Kuberskaya 1980, 1984, 1988).

The number of chromosomes in the set is eight. This is the usual situation for the majority of described chironomid species (Petrova 1989). A small heterozygous inversion is present in chromosome II in all the specimens studied of *L. willasseni*. The number of transcriptionally active regions identified is small: one Balbiani ring in chromosome I and one nucleolus in chromosome IV. In contrast to the previously described *Diamesinae*, the homologes of the large polytene chromosomes are often unpaired in few regions and they have a meandric structure. Because few studies of *Diamesinae* karyosystematics have been conducted to date, the full significance of the patterns described above is presently unclear. Additional representatives of *Lappodiamesa* and related genera should therefore be examined for further comparison.

Acknowledgements

We are grateful to Dr E. Willassen from Bergen University for help in preparing the manuscript.

References

Bauer, H. 1936. Beiträge zur vergleichende Morphologie der Speicheldrüsenchromosomen (Untersuchungen an den Riesenchromosomen der Dipteren II.—*Zool. J. Physiol. 56:* 239–276.

Kieffer, J. J. 1922. Chironomides de la Nouvelle-Zemble.—*Rep. sc. Res. Norw. Exped. Novaya Zemlya 1921 2:* 1–24.

Kuberskaya, E. F. 1974. On the characteristics of the karyotype and some special features of the nucleus structure in the *Pseudodiamesa nivosa* from the Lake Baikal area.—*Cytology 16:* 1426–1432.

Kuberskaya, E. F. 1979. Karyotypical characteristics of chironomids, subfamily Diamesinae (Diptera, Chironomidae) from Baikal area. In *Karyosystematics of invertebrate animals* (ed. L. A. Chubareva): 47–50. Zoological Institute Press, Leningrad, Nauka.

Kuberskaya, E. F. 1980. *Morphology of salivary glands and karyotypical characteristic Diamesinae (Diptera, Chironomidae) from Baikal territory*. Irkutsk University Press, Irkutsk.

Kuberskaya, E. F. 1984. The morphology of salivary glands and karyotypical characteristic of *Diamesa tsutsuii* Tok. (Diptera, Chironomidae) from Primorye. In *The biology of freshwater animals of the Soviet Far East* (ed. I. M. Levanidova): 102–106. DVNC AN SSSR Press, Vladivostok.

Kuberskaya, E. F. 1988. Morphological characteristic of salivary glands and peculiarity of the karyotype of *Diamesa leona* Rob. (Diptera, Chironomidae) from Sachalin Island. In *Fauna, systematics and biology of freshwater invertebrates* (eds I. M. Levanidova & E. A. Makarchenko): 36–39. DVNC AN SSSR Press, Vladivostok.

Makarchenko, E. A. 1983. On systematics and distribution of *Lappodiamesa brundini* Serra-Tosio (Diptera, Chironomidae). In *Ecology and systematics of freshwater organisms from Far East* (eds I. M. Levanidova & E. A. Makarchenko): 52–56. DVNC AN SSSR Press, Vladivostok.

Makarchenko, E. A. 1985. *Chironomids of the Soviet Far East. Subfamilies Podonominae, Diamesinae and Prodiamesinae (Diptera, Chironomidae)*. DVNC AN SSSR Press, Vladivostok.

Martin, J. 1979. Chromosomes as tools in taxonomy and phylogeny of Chironomidae (Diptera).—*Ent. scand. Suppl. 10:* 67–74.

Petrova, N. A. 1983. Karyotype and unstable associations of polytene chromosomes in *Syndiamesa nivosa* (Diptera, Chironomidae).—*Zool. Zh. 62:* 74.

Petrova, N. A. 1986. Importance of karyological features for taxonomy, systematics and evolution of chironomids. In *Evolution, species formation and systematics of chironomids* (eds N. N. Kolesnikov & A. G. Istomina): 29–35. Institute of Cytology and Genetics SO AN SSSR Press, Novosibirsk.

Petrova, N. A. 1989. Characteristics of chironomid karyotypes (Diptera, Chironomidae) of world fauna. I. Subfamilies Telmatogetoninae, Podonominae, Tanypodinae, Diamesinae, Prodiamesinae and Orthocladiinae.—*Entomol. obozr. 68:* 107–120.

Saether, O. A. 1980. Glossary of chironomid morphology terminology (Diptera, Chironomidae).—*Ent. scand. Suppl. 14:* 1–51.

Saether, O. A. and Willassen, E. 1988. A review of *Lappodiamesa* Serra-Tosio, with the description of *L. boltoni* spec.nov. from Ohio, U.S.A.—*Spixiana, Suppl. 14:* 75–84.

Serra-Tosio, B. 1968. Taxonomie phylogenetique des Diamesini: les genres *Potthastia* Kieffer, *Sympotthastia* Pagast, *Parapotthastia* n.g., et *Lappodiamesa* n.g. (Diptera, Chironomidae).—*Trav. Lab. Hydrobiol. piscic. Univ. Grenoble 50–60:* 117–164.

Wülker, W. and Götz, P. 1968. Die Verwendung der Imaginal-scheiben zur Bestimmung des Entwicklungszustandes von *Chironomus*-Larven (Diptera).—*Ztschr. Morphol. 62:* 362–388.

Zacharias, H. 1984. Allocyclic behaviour and underreplication of the nucleolus chromosome in *Pseudodiamesa* (Chironomidae).—*Chromosoma 89:* 263–273.