Some New Species of *Dactylogyrus* from the European Freshwater Fishes

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Abstract. On the basis of investigations on the representatives of the genus *Dactylogyrus* (*Monogenoidea*) parasitizing the European freshwater fishes it was ascertained that some species of these parasites form groups of two or more closely related species. The author presents descriptions, figures and differential diagnoses of 7 new species: *Dactylogyrus rarissimus* on *Rutilus rutilus*, *D. izjumovae* on *Scardinius erythrophthalmus*, *D. nanoides* on *Leuciscus cephalus*, *D. dirigerus* and *D. elegantis* on *Chondrostoma nasus*, *D. haplogonoides* on *Vimba vimba*, *D. volgensis* on *Blicca bjoerkna* and *D. finitimus* on *Gobio gobio*.

The paper also contains descriptions and figures of determinating characters of the species *Dactylogyrus suecicus*, *D. distinguendus* and *D. micracanthus*, which were very often mistaken for morphologically closely related species or regarded as their developmental stages.

The author then pays attention to polymorphism which has been observed in populations of the species *Dactylogyrus ergensi*, *D. crucifer*, *D. zandti* and *D. distinguendus* and which may be considered as one of the phases of the origin of species and as the formation of pairs or groups of closely related parasite species on one or more closely related host species.

In the form of a footnote the author draws attention to and takes up a negative standpoint towards the discovery of the so-called 8th pair of marginal hooks in the representatives of *Dactylogyridae* as described by the American authors (MIZELLE and PRICE 1963, PRICE and MIZELLE 1964).

After studying the parasite fauna of fishes in the Tisa river basin (July 1962, July-August 1963, July 1964) and after examining the material collected in previous years some new species of the genus *Dactylogyrus* were discovered. It was established that grave errors occurred in the identification of some widespread and seemingly well-known species of *Dactylogyrus* due to incomplete and sometimes inaccurate systematic descriptions and drawings. Different species were often confused with one another, were mistaken for others or were considered to be developmental variants of one species. These errors were repeated and still aggrevated in cursory determinations, causing confusion and further mistakes in the conclusions on the occurrence and distribution of individual species of parasites

on various hosts, as well as in the elucidation of the infection rate or the seasonal and developmental dynamics of the parasite fauna of various fish species.

After some uncharacteristic parasites were included in the list of parasite fauna of hosts when single accidental findings were made and because the causes of this deviation from the usual specificity were not analyzed when these findings repeated themselves, many investigators were also led astray.

Unfortunately a number of such errors were included in some revisions such as "The fish parasites of the Ukrainian SSR" by A. P. MARKEVICH (1951), "The key of parasites of freshwater fishes of the USSR" edited by B. E. Bykhovsky (GUSSEV, 1962) etc. The present paper and other communications (MOLNAR, 1964a, b, GUSSEV 1965, GLÄSER and GUSSEV, in press) bring considerable changes in previous concepts on many current species of *Dactylogyrus*, which on many occasions have been proved to form groups. Apart from this, some previously described species which were unjustly listed as synonyms of other species, have been restored as distinct species. In this connection all previous data had to be revised.

The species described in this paper were studied on live material and on constant glycerin-gelatine preparations with the employment of phase contrast microscopy.

The types and paratypes of the new species are deposited in the collections of the Zoological Institute, the USSR Academy of Sciences (Leningrad).

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Dactylogyrus rarissimus n. sp. (Fig. 1)

(syn. D. sp. Ergens, 1962)

A very small worm up to 0.25 long, 0.11 mm wide. The attaching disc (haptor) delimited from the body proper by a slight constriction. The marginal hooks with well defined elongated base ("handle") and shaft and with projecting "opposable piece" of the hook point; length 0.015—0.028 mm, smallest hooks are those of the 6th and 7th pair.*) The middle hooks (anchors) are slender, with well developed

*) The discovery of the 8th pair of marginal hooks (MIZELLE and PRICE, 1963, PRICE and MIZELLE, 1964) in *Dactylogyrus*, which was a sensational finding to the *Monogenoidea* specialists, is not discussed in detail now, as it will be given special attention in a separate paper. However, it is necessary to note the following: 1. MIZELLE and PRICE consider as hook not an independent, but "auxiliary" formation which is apparently a ligament, working the hook back by its "opposable piece". The same function is attributed to the second ligament which is slightly different in its structure, pulling the hook in the opposite direction and deviating from the opposite convex side of the hook point ("the posteriorly projecting structure" according to the American authors). The latter is met with in all marginal hooks, while the newly "discovered" one is found only in the 6th pair of hooks (according to the numeration after KULWIEC, 1927). 2. The form of this ligament which is depicted as a hook in the drawings by MIZELLE and PRICE, does not agree with the reality. In fact it is shaped like a thin thread gradually tapering to the point of the 6th pair of hooks with a thickened pear-shaped opposite end and flanked by two thin control cords which come up to its both ends. 3. The discussed formation was discovered by the author in 1948—1949, and under the name "little liga-

291 roots and sickle-shaped point; total length 0.027—0.032 mm; length of the anchor base (shaft) 0.022—0.025 mm, of the outer root 0.003—0.005 mm, of the inner root 0.009—0.010 mm, of the point 0.010—0.011 mm. The main connecting (dorsal) bar is bent backwards in its central part, with posteriorly directed ends and measures about 0.003 × 0.020 mm. The supplementary (ventral) bar is ⊥-shaped resembling the letter T upside down or the bar of the type *D. crucifer*. It consists of three appendages which branch from a point of junction and of which the two posterior ones are drawn together and the anterior one stands apart. Its total measurements are 0.010—0.014 × 0.011—0.019 mm. the length of anterior appendage about 0.008 mm.

The copulatory organ consists of a tube and a supporting piece. Its total length is 0.016—0.020 mm. The tube is short, wide, with expanded initial part, cylindrical in further length, with obliquely cut end; its length is about 0.015 mm, the diameter of initial part 0.004—0.005 mm, of the remaining part about 0.0016 mm. The vaginal chitinoid armature was not detected.

Location, host and localities: the gill filaments of *Rutilus rutilus*; the Volga river delta (collections in 1952), the Latorica river, the White Lake (Beloye ozero, collections in 1931), the Aral Sea.

The species is closely related to *D. crucifer* Wagener, 1857, differing from it in the more minute chitinoid formations, the shape of supplementary bar of haptor and in the structure of the copulatory organ.

Dactylogyrus izjumovae n. sp. Fig. 2.

A very small worm up to 0.39 mm long, 0.10 mm wide. The haptor delimited from the body proper by a slight constriction. The marginal hooks with well defined base and shaft and projecting "opposable piece" of the point. Their length varies between 0.015—0.025 mm; the smallest hooks are those of the 7th pair. The anchors with well developed roots and sharply curved, nearly straight, considerably long point. Their total length ranges from 0.026 to 0.031 mm, the length of the anchor base 0.019—0.023 mm, of the outer root 0.003 mm, of the inner root 0.011—0.012 mm,

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ments" was mentioned in the text and depicted in the drawings representing the haptoral armature of larvae and some adult forms (Gussev, 1955, pp. 228, 243, 276, Fig. 4, 20, 25, 37; Gussev, 1962, Fig. 605, 664, 678, 699 and other papers still in press). The study of this formation in different species with the employment of phase microscopy has lately helped to specify its structure and confirmed the author's original opinion about its dependence upon and relevancy to the 6th pair of hooks, as in the case of all other ligaments of marginal hooks and anchors in *Dactylogyrus*, *Ancyrocephalus*, *Ancylodiscoides* etc. Therefore, the presence of 7 pairs of marginal hooks should still be considered a characteristic feature in the genus *Dactylogyrus* as well as in all members of *Dactylogyridae*. In all species the ligaments are lacking in variety, only sometimes they differ in thickness and strength. This is also the reason why they are usually being omitted in drawings and descriptions, the more so because in preparations they are not always visible. The discovery of two "additional" pairs of marginal hooks in *Ancyrocephalinae* (Mizelle and Price, 1965) is apparently just as groundless.

of the point 0.012 mm. The main connecting bar is slightly bent backwards in its central part, of equal thickness in its total length, with slightly curved ends posteriorly and measures 0.003 × 0.023 mm. The supplementary bar is ⊥-shaped, with a broad onwards expanded appendage and slender lateral appendages between which a slit juts out; the bar measures 0.09—0.010 × 0.019—0.021 mm.

The copulatory organ consists of a tube and a supporting piece; its total length ranges from 0.023—0.033 mm. The tube is thin and long, coiled in spiral (making 1.5 turn), with bladder-shaped initial section and its length is 0.060—0.070 mm; the initial part measures about 0.010 — 0.007 mm, the diameter of the central

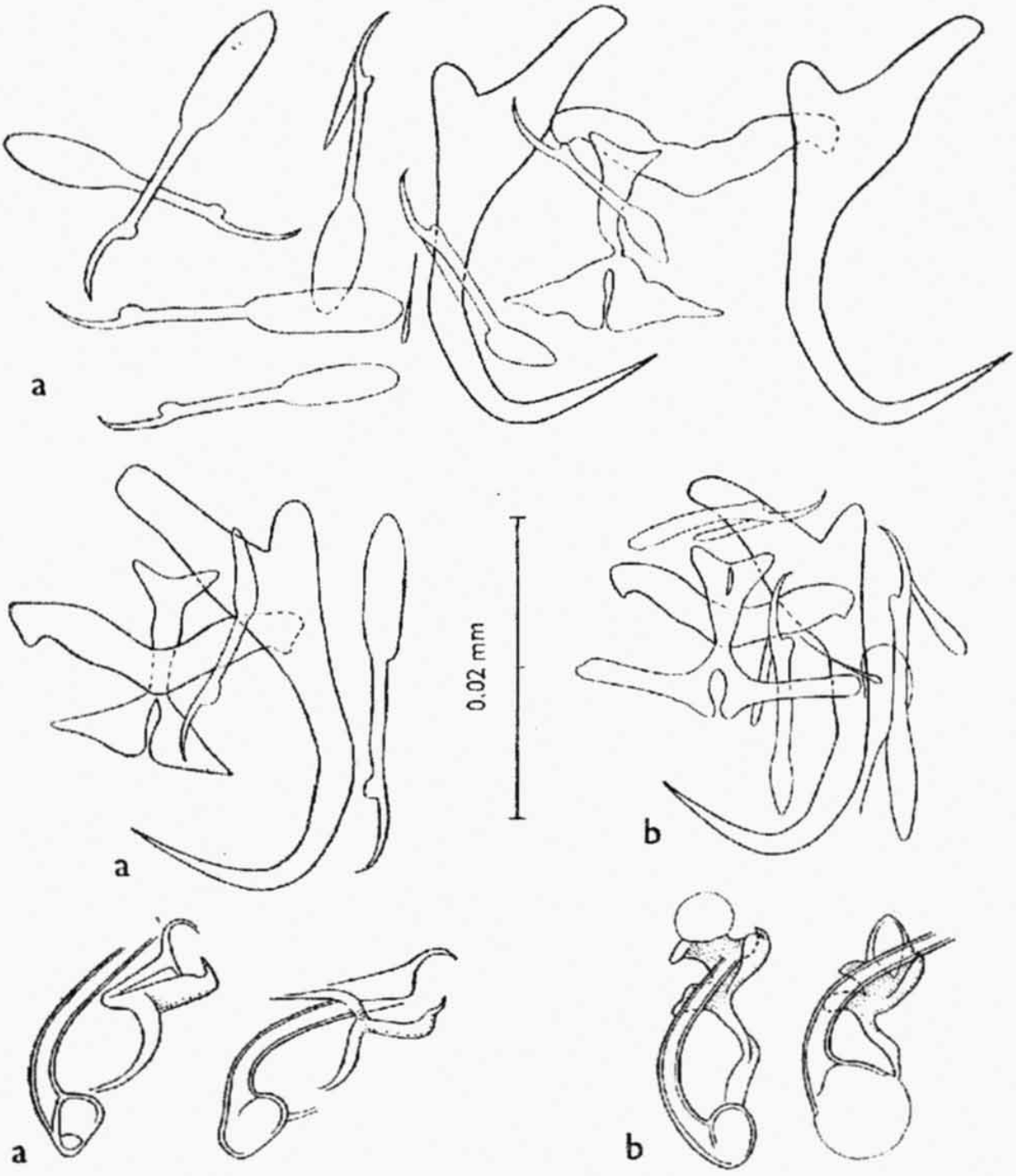

Fig. 1. *Dactylogyrus rarissimus* n.sp.: a – from the Volga delta, b – from the Seliger Lake

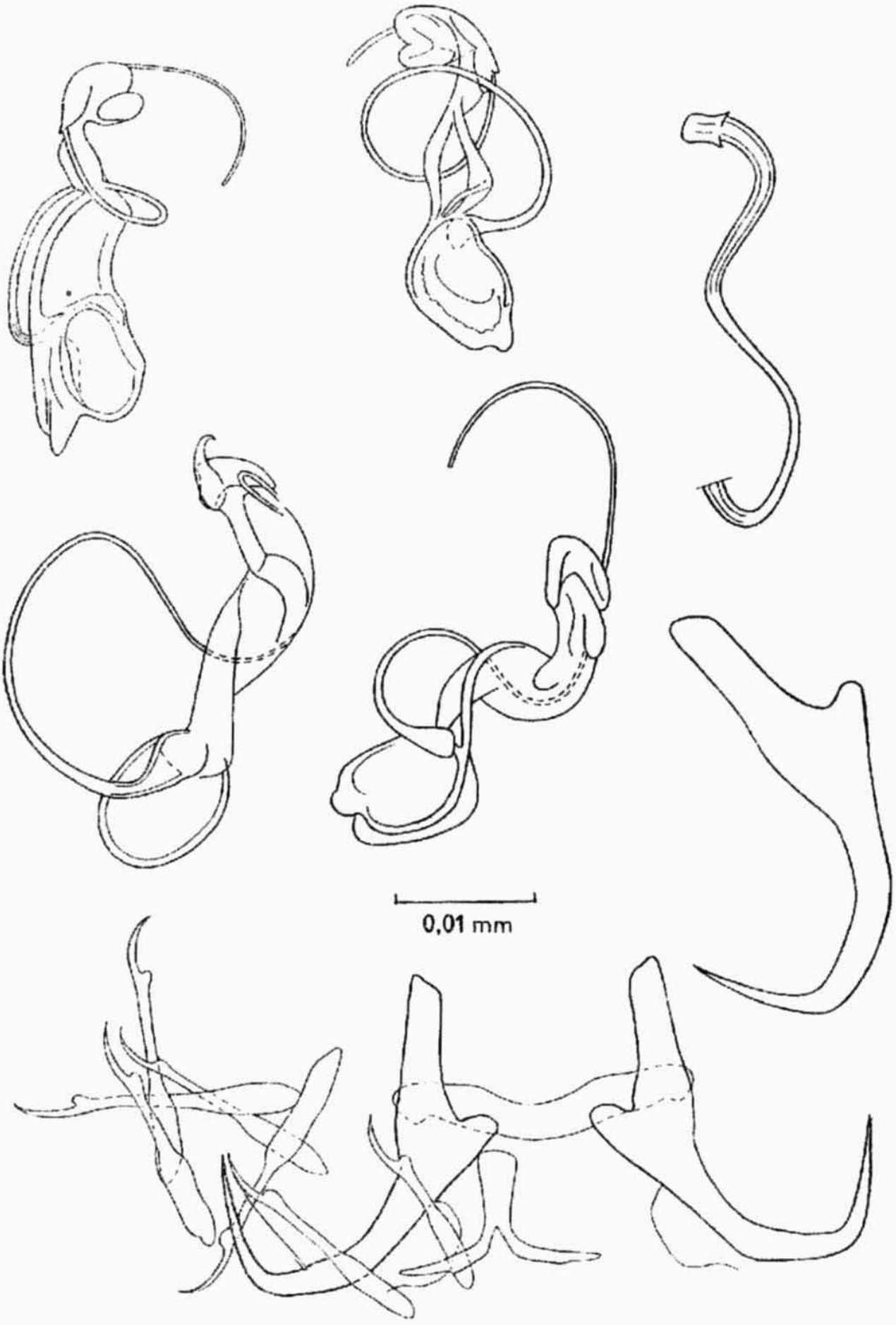

Fig. 2. *Dactylogyrus izjumovae* n.sp.

part less than 0.001 mm. The supporting piece appears as a ribbon-shaped plate with thickenings connected with the initial part of tube. Its terminal part has bolster- forming thickenings; length of supporting piece is 0.015—0.023 mm. The vagina has the shape of a long, coiled tube, its length being between 0.039 mm and 0.045 mm, the diameter about 0.002 mm (with one end twice as thick).

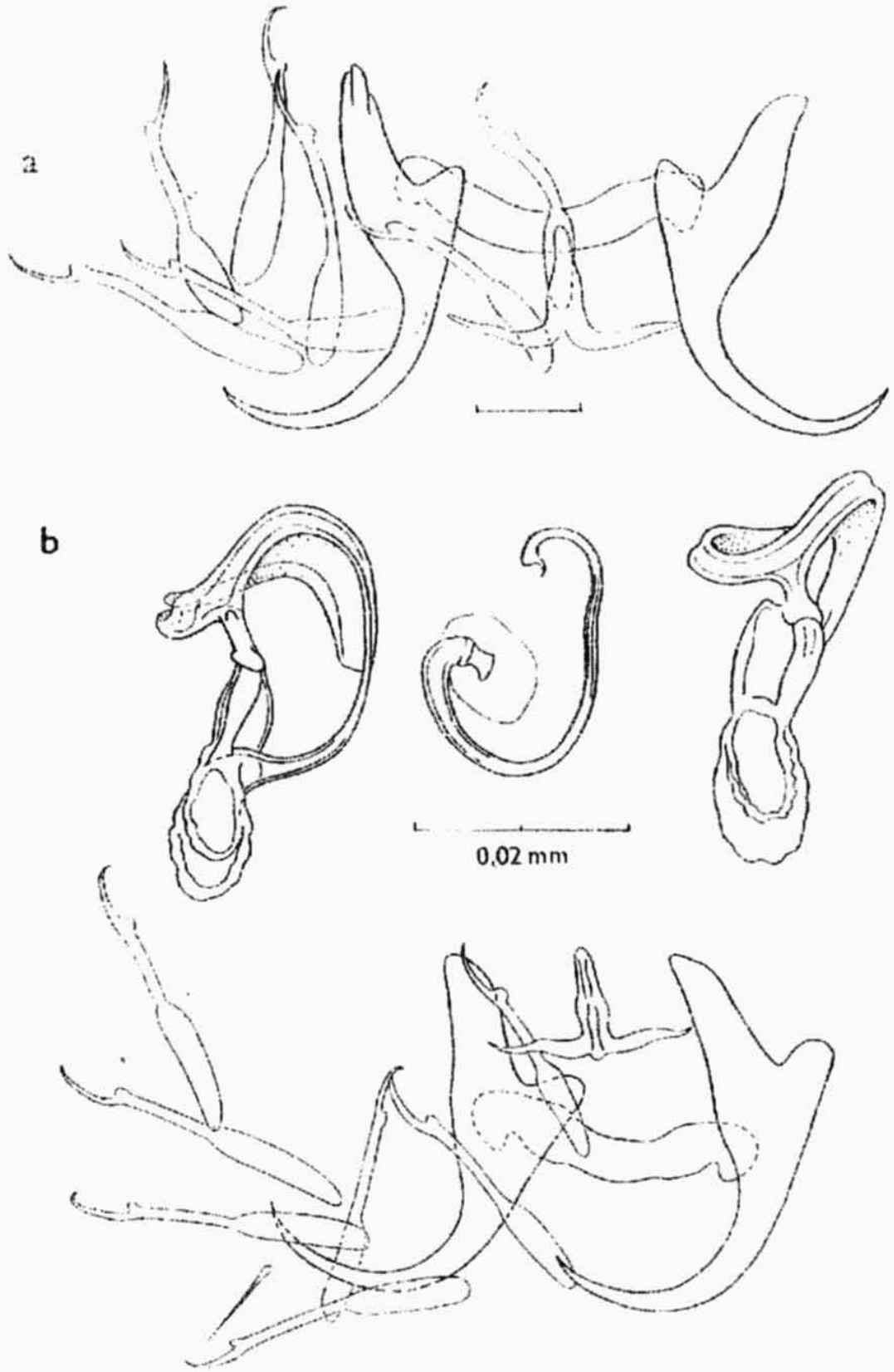

Fig. 3. *Dactylogyrus dirigerus* n.sp.: a—from the neighbourhood of Batumi, b—from the Tisa river

Location, host and localities: gill filaments of *Scardinius erythrophthalmus*; river Latorica (near the town Čop).

The species was named in honour of the author's colleague N. A. Izjumova.

9 specimens were investigated.

In its chitinoid formations of haptor the species *D. izjumovae* resembles *D. difformis* Wagener, 1857, but they are smaller in the former than in the latter. In its structure the copulatory organ is very peculiar, slightly resembling that of *D. zandti* Bychowsky, 1933.

Dactylogyrus dirigerus n. sp. (Fig. 3)

A small or medium size worm up to 0.53 mm long, 0.11 mm wide. The haptor slightly delimited from the body. The marginal hooks with well defined base and shaft and a rounded projecting "opposable piece" of the point; their length 0.023 to 0.035 mm; smallest hooks are those of the 6th and 7th pair. The anchors with well developed roots and a rather straight point only slightly definable from the anchor base. Their total length is 0.033—0.038 mm, length of the anchor base 0.027 to 0.030 mm, of the outer root 0.003—0.004 mm, of the inner root 0.012 to 0.015 mm. The connecting bar is bent backwards in its central part, with posteriorly directed extremities; it measures 0.003—0.006 × 0.024—0.030 mm. The supplementary bar is ⊥-shaped and measures 0.009—0.012 × 0.020—0.024 mm.

The copulatory organ consists of a tube and a supporting piece. Its total length ranges between 0.037—0.042 mm. The thin copulation tube is curved in an arch, with bladder-like initial part; its terminal part disappears in the folds of the supporting piece; the diameter in its middle is less than 0.001 mm, the initial part measures 0.013—0.015 × 0.008 to 0.009 mm. The supporting piece starts from the initial part in the shape of a wide plate and soon forms a projection, gradually changing into a sickle-shaped form which meets the tube thus forming a ring. The vaginal tube is long, curved, somewhat expanded at one end; its length is 0.046—0.053 mm, the diameter 0.001 mm (in central part) — 0.003 mm (at the wide end).

Location, host, localities: gill filaments of *Chondrostoma nasus* L., also found on *Ch. colchicum* (Kessler); river Tisa, and near Batumi.

15 specimens were investigated.

The species is closely related to *D. ergensi* Molnar, 1964, differing from it in the form of its anchors: the point smoothly extends into the anchor base, while in the latter species the point is distinctly demarcated from the anchor base by a sharp bend.

Dactylogyrus elegantis n. sp. (Fig. 4)

A small worm up to 0.30 mm long, 0.04 mm wide. The marginal hooks with well defined base and shaft and a rounded, projecting "opposable piece" of the

point; their length ranges from 0.015—0.023 mm, the shortest hooks being those of the 7th pair, the longest — of the 4th pair. The anchors have a sabre-shaped straight point which imperceptibly passes into the anchor base; their roots are well developed. Their total length is 0.024—0.028 mm, the length of the anchor base including the point 0.022—0.025 mm, of the outer root 0.002—0.004 mm, of the inner root 0.006—0.010 mm. The connecting bar is bent backwards in its central part, with expanded and posteriorly pointed ends: it measures 0.002 to

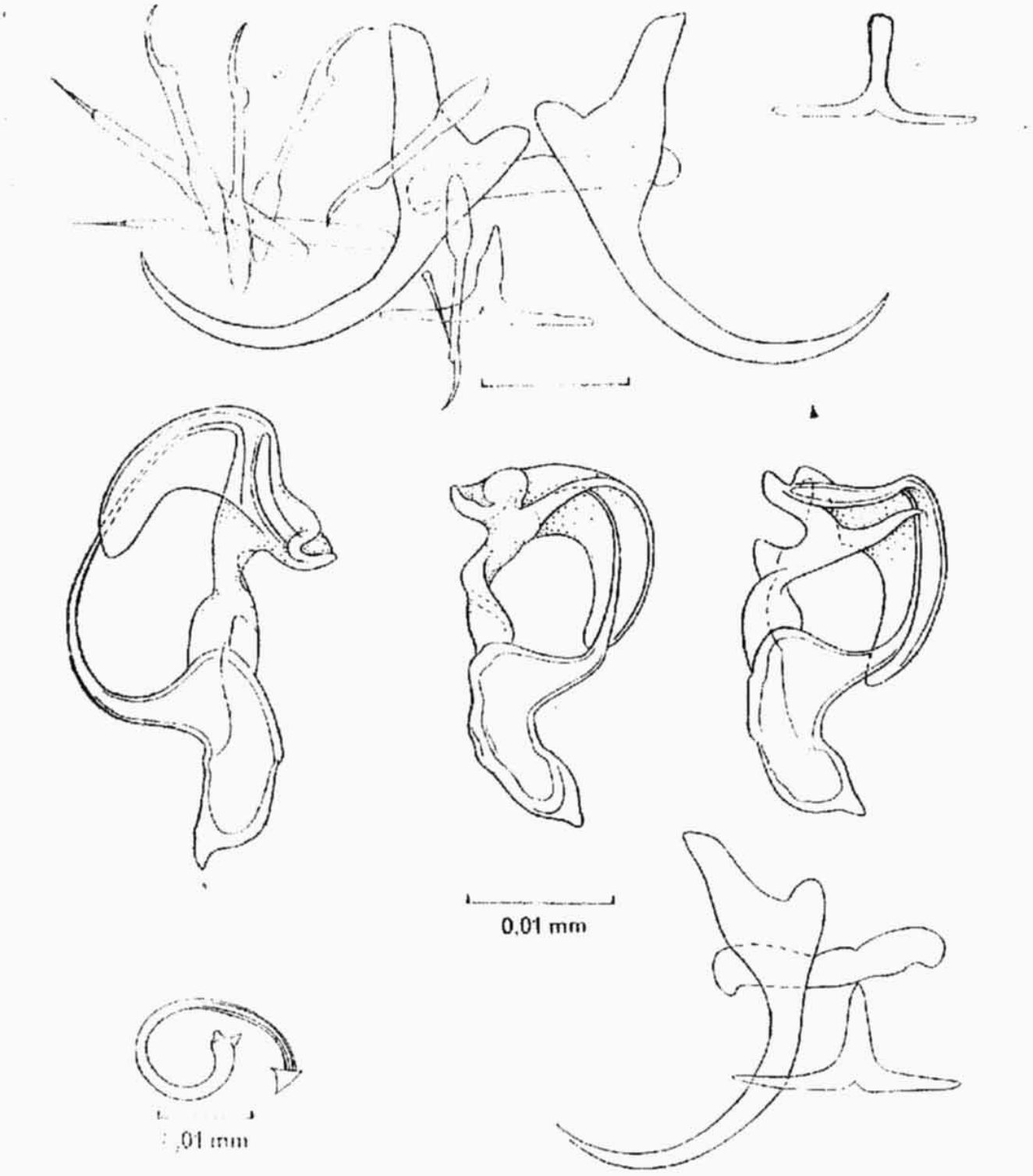

Fig. 4. *Dactylogyrus elegantis* n.sp.

297 0.003 × 0.016—0.019 mm. The supplementary bar is ⊥-shaped, with the anterior appendage tapering to tip, and measures 0.007 × 0.015—0.017 mm.

The copulatory organ consists of a tube and a supporting piece. Its total length varies between 0.025—0.031 mm. The tube is curved in the form of a ring, is thin, with expanded kidney-shaped initial part. The initial part measures about 0.014 × 0.005 mm, the diameter of tube in the middle is less than 0.001 mm. The supporting piece starts from the initial part of tube in the shape of a broad plate which, after getting thicker, extends into a groove-like, curved part covering the tube. The vaginal armature has the shape of a curved tube, with a characteristic triangular cap; its length is about 0.038 mm, the diameter 0.001—0.002 mm.

Location, host, locality: gill filaments of *Chondrostoma nasus*. River Tisa. 10 specimens were investigated.

This species is closely related to *D. dirigerus*, differing from it in thinner and fine anchors and other parts of the haptoral armature, in smaller size of the copulatory organ and vaginal tube and in the structural details of the copulatory organ, particularly in longer initial part of its tube.

D. elegantis was not found in 1962 and during the warm period of the summer 1963. Its occurrence started since the middle of August 1963, when the water temperature in the river dropped.

Dactylogyrus ergensi **Molnar, 1964** (Figs. 5, 6)

(Syn. *D. nybelini* Markewitsch in ERGENS, 1959a, part; *D. chondrostomi* Malewitzkaja in KULAKOWSKAJA, 1960, part).

This species was found in large numbers on the gills of *Chondrostoma nasus* in the river Tisa. It was previously reported from the same host from the rivers Prut and Dnestr and from the water reservoirs of Czechoslovakia. It was recently detected by V. V. Kashkowsky in the Iriklin water reservoir (river Ural) and by T. Mikailov in the Kura river basin.

The analysis of the data from literature and of the available material brings evidence on a considerably wide range in the fluctuations of the sizes of chitinoid formations in different populations of the species (Table 1, Figs. 5, 6).

According to the material obtained from the river Tisa by ERGENS (1959), LUCKÝ (1957) and the author himself, the species could be divided into two distinct forms, differing from each other in the length of anchors — this being either 0.038 to 0.045 mm or 0.029—0.034 mm.

But according to the data of KULAKOWSKAYA (1960) and also according to the materials kindly loaned by KASHKOVSKY (from the Iriklin water reservoir) and by ERGENS (from Czechoslovakia) the distinction between these "forms" disappears and a continuous series presents itself.

However, it must be said that during the investigations carried out at the Tisa river in July and August of 1962 and 1963 two mentioned "forms" of *D. ergensi*

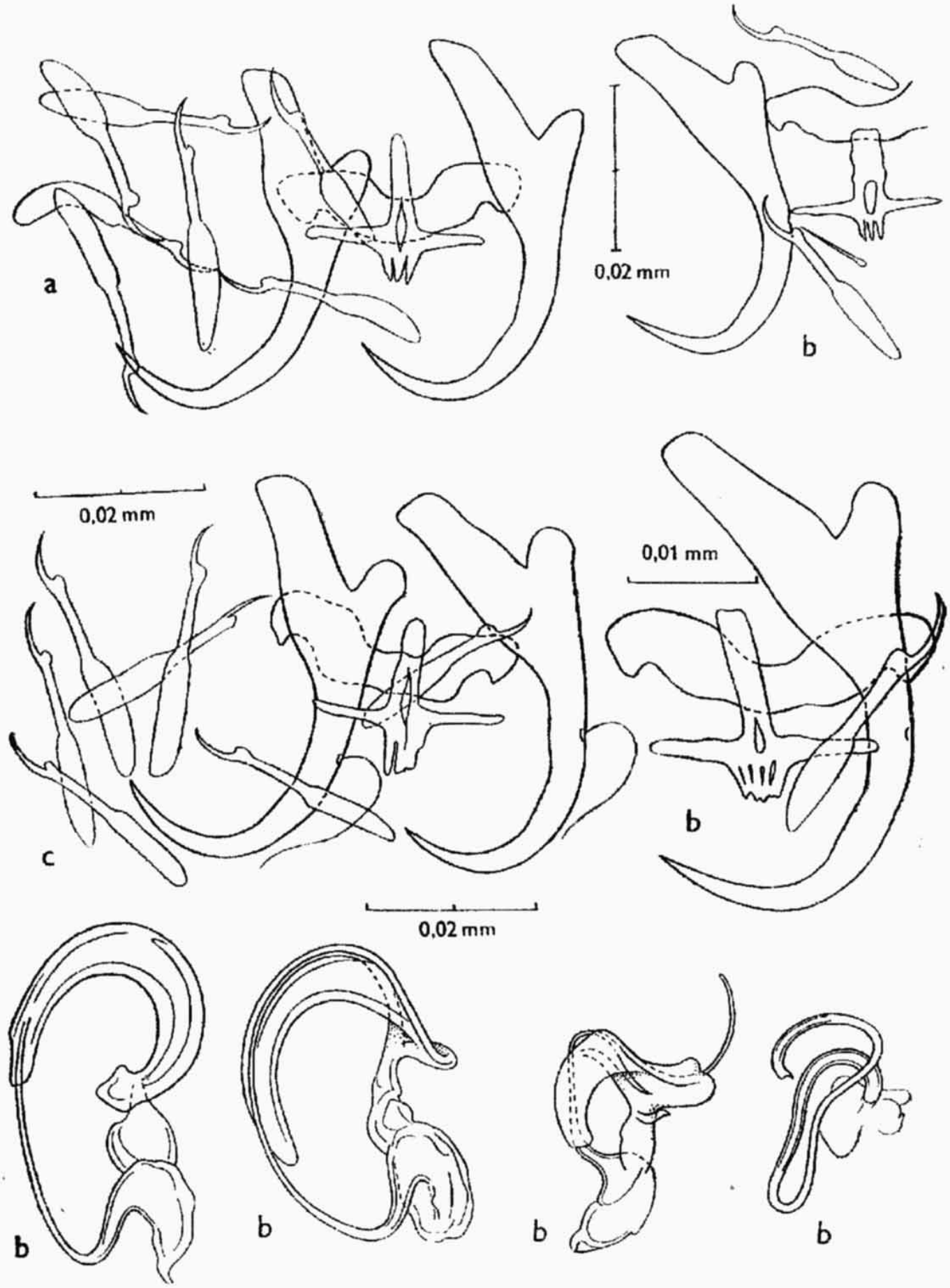

Fig. 5. *Dactylogyrus ergensi* Molnar, a form with large chitinoid formations: a from the Volga river, b—from the Tisa river, c—from the Prut river

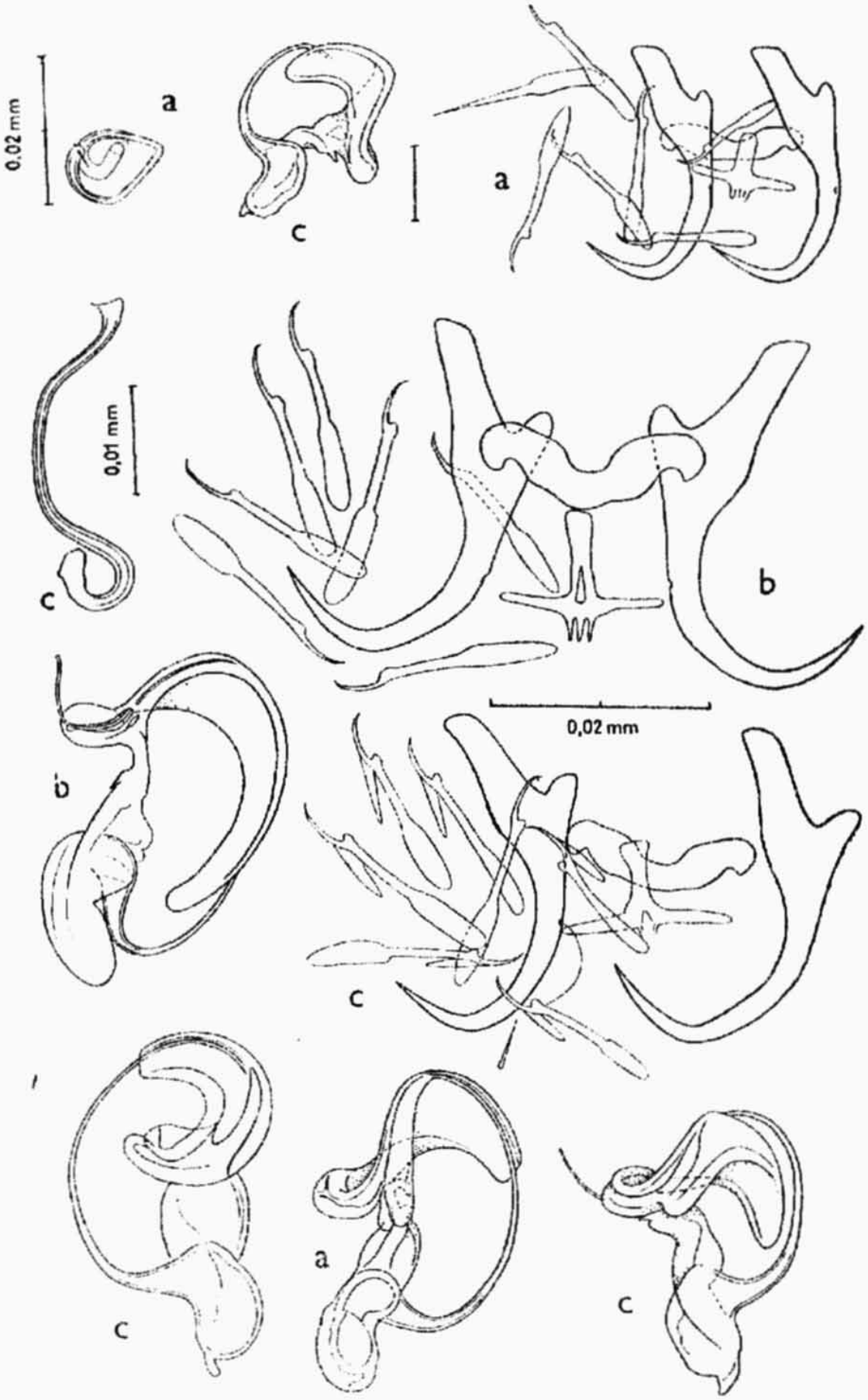

Fig. 6. *Dactylogyrus ergensi* **Molnar, a form with small chitinoid formations: a—from the Prut river, b—from the Lyukavitsa river (Tisa basin), c—from the Tisa river**

were found among the population of *D. ergensi* and other species of *Dactylogyrus* collected from the gills of 25 specimens of *Chondrostoma nasus*. There is no distinct line between them, but still some incongruity can be traced. No differences in the topology of these forms could be noticed on the gills.

Dactylogyrus volgensis n. sp. (Fig. 7)

A small worm up to 0.4 mm long, 0.10 mm wide. The marginal hooks with a thickened base and a projecting "opposable piece" of the point, their length ranging from 0.017—0.026 mm. The anchors are stout and massive, with well developed roots and curved point. Their total length is 0.030—0.031 mm, the length of the anchor base 0.025—0.027 mm, of the outer root 0.005 mm, of the inner root 0.012 to 0.013 mm, of the point 0.010 mm. The connecting bar is slightly bent with somewhat expanded beak-like extremities and measures 0.003 × 0.027 mm. The supplementary bar is ⊥-shaped, with a short anterior appendage and measures 0.005 × 0.016—0.018 mm.

The copulatory organ consists of a tube and a supporting piece. The tube is bent almost in the right angle with a bladder-like initial part tapering gradually to end; its length (along the bend) is 0.030 mm, the diameter of the initial part about 0.008 mm, in the middle 0.002 mm.

The supporting piece is in the shape of a folded formation, with expanded anterior part and connected with the initial part of tube by means of a thick ribbon-like

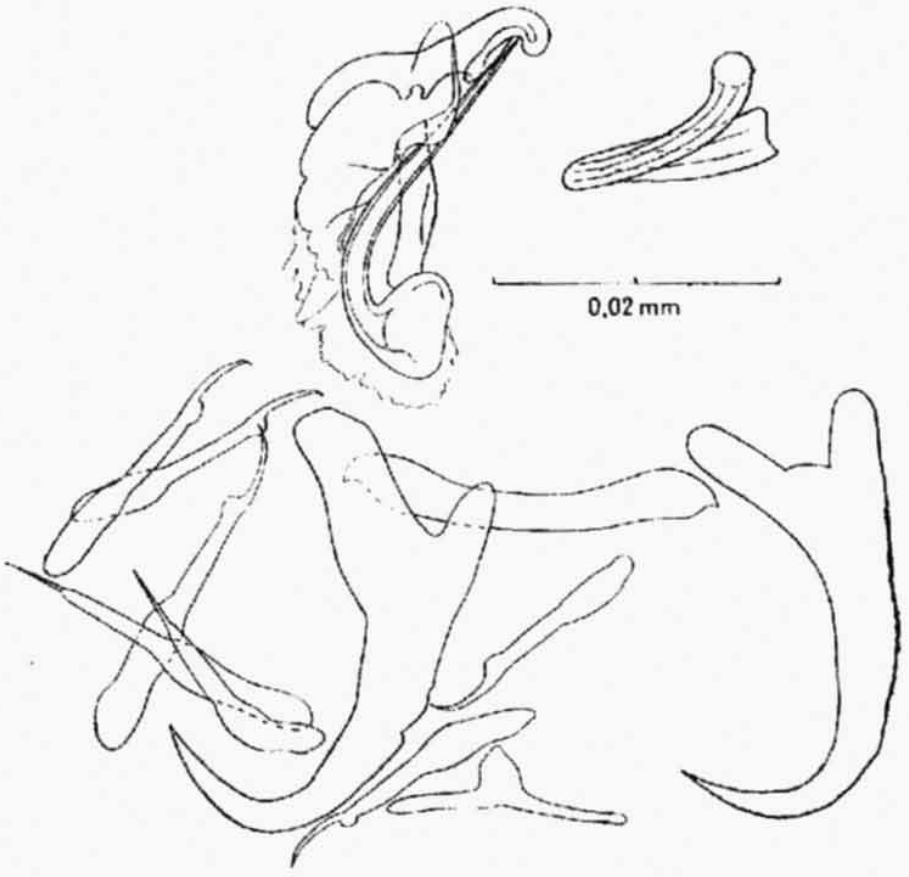

Fig. 7. *Dactylogyrus volgensis* n.sp.

Table 1. Correlation of sizes of the chitinoid formations in various forms of *D. ergensi* (in mm)

Formation	"Large" form			"Small" form		"Medium" form
	Ergens 1959, material from South Bohemia	Molnar 1964, material from the Danube	Gussev, material from the Tisa and Volga rivers	Lucký 1957, material from the Svitava river	Gussev, material from the Tisa river	Ergens, material from various water reservoirs of the Odra and Danube river basins, Czechoslovakia, also from material collected by Kashkovsky
Anchors						
total length	0.041–0.043	0.040–0.042	0.038–0.045	0.033–0.034	0.029–0.033	0.033–0.043
length of anchor base	0.032–0.034	0.031–0.033	0.029–0.036	0.028–0.029	0.023–0.026	0.023–0.033
length of outer root	0.004–0.006	0.005–0.007	0.003–0.005	0.003	0.003	0.003–0.004
length of inner root	0.015–0.017	0.016	0.012–0.015	0.010–0.012	0.010–0.012	0.010–0.013
length of point	0.013–0.015	0.012	0.011–0.014	0.008	0.009–0.011	0.010–0.012
Connecting bar						
length	0.005–0.007	0.005–0.006	0.003–0.006	0.005	0.002–0.004	0.004–0.005
width	0.026	0.029–0.031	0.023–0.030	0.019–0.020	0.018–0.020	0.023–0.024
Supplementary bar						
lenght	0.015–0.017	0.013–0.017	0.012–0.019	—	0.009–0.011	0.012–0.013
width	0.021–0.023	0.014–0.017	0.018–0.022	—	0.016–0.018	0.020
Marginal hooks						
length	0.024–0.028	0.025–0.032	0.024–0.031	0.018–0.024	0.019–0.026	0.019–0.027
Copulatory organ						
length	0.032–0.040	0.041–0.057	0.030–0.050	0.036	0.025–0.037	0.030–0.045
Vaginal tube						
length	0.064	0.024–0.028 (?)	0.064–0.085	—	0.039–0.060	0.045–0.060

shaft. The vaginal armature is a wide V - shaped tube (or straight?), 0.032 mm long and with a diameter between 0.002—0.003 mm.

Location, host and locality: gill filaments of *Blicca bjorkna*; the river Volga delta (Tumak).

2 specimens were investigated.

D. volgensis differs from all other species possessing the ⊥-shaped supplementary bar in rough and massive anchors, short anterior arm of the supplementary bar and in the structure of the copulatory organ.

Dactylogyrus nanus Dogiel et Bychowsky, 1934 (Fig. 8)

(syn.: *D. gemellus* Nybelin, 1936)

A small worm up to 0.40 mm long, 0.10 mm wide. The haptor slightly delimited from the body by a constriction. The marginal hooks with a base distinctly discernible from the shaft and with a rounded, projecting "opposable piece" of the point; their length varies between 0.016—0.025 mm, the longest hooks being those of the 2nd and 3rd pair, the shortest of the 7th pair. The anchors with well developed roots, a small, regularly and sharply curved point and with a relatively short, expanded section of the anchor base (the author proposes to name this characteristic form of anchors "nanus type"). Their total length is 0.026—0.032 mm, the length of the anchor base 0.024—0.028 mm, of the outer root 0.002 —0.004 mm, of the inner root 0.004—0.007 mm, of the point 0.005—0.006 mm. The connecting bar is slightly bent backwards in the middle, with expanded and sharply curved extremities and measures 0.002—0.004 × 0.016—0.021 mm. The supplementary bar is ⊥-shaped with a stout anterior appendage which has a slit inside and expands towards a concave, straight or convex end. The bar measures 0.008 to 0.010 × 0.013—0.020 mm.

The copulatory organ consists of a tube and a supporting piece. The tube is sickle-shaped with almond-like initial part, tapering to the end at first abruptly, then gradually. The supporting piece starts from the beginning of the initial part of tube in the form of a ribbon, with a characteristic thickening in the middle of the formation, which extends into a spade-like expanded triangular end with characteristic finger-like and claw-like appendages to the right and to the left. The total length of the copulatory organ is 0.024—0.029 mm, the length of tube along the bend 0.025—0.035 mm, the diameter in the middle about 0.001 mm, the initial part measures 0.012—0.014 × 0.005—0.006 mm. The supporting piece is 0.012—0.016 mm long. The vaginal tube is shaped as a wide formation with a cap at the end, is more or less arch-like bent, its length is about 0.008—0.015 mm, the diameter 0.002—0.003 mm.

Location, host and distribution: gill filaments of *Rutilus rutilus*; is found wherever its host or its subspecies are widespread.

303 Large material was investigated from the water reservoirs of Czechoslovakia, from the rivers Tisa, Volga, Ural, the Aral Sea and the Seliger Lake.

This species is found only on *Rutilus rutilus*. All data about its occurrence on

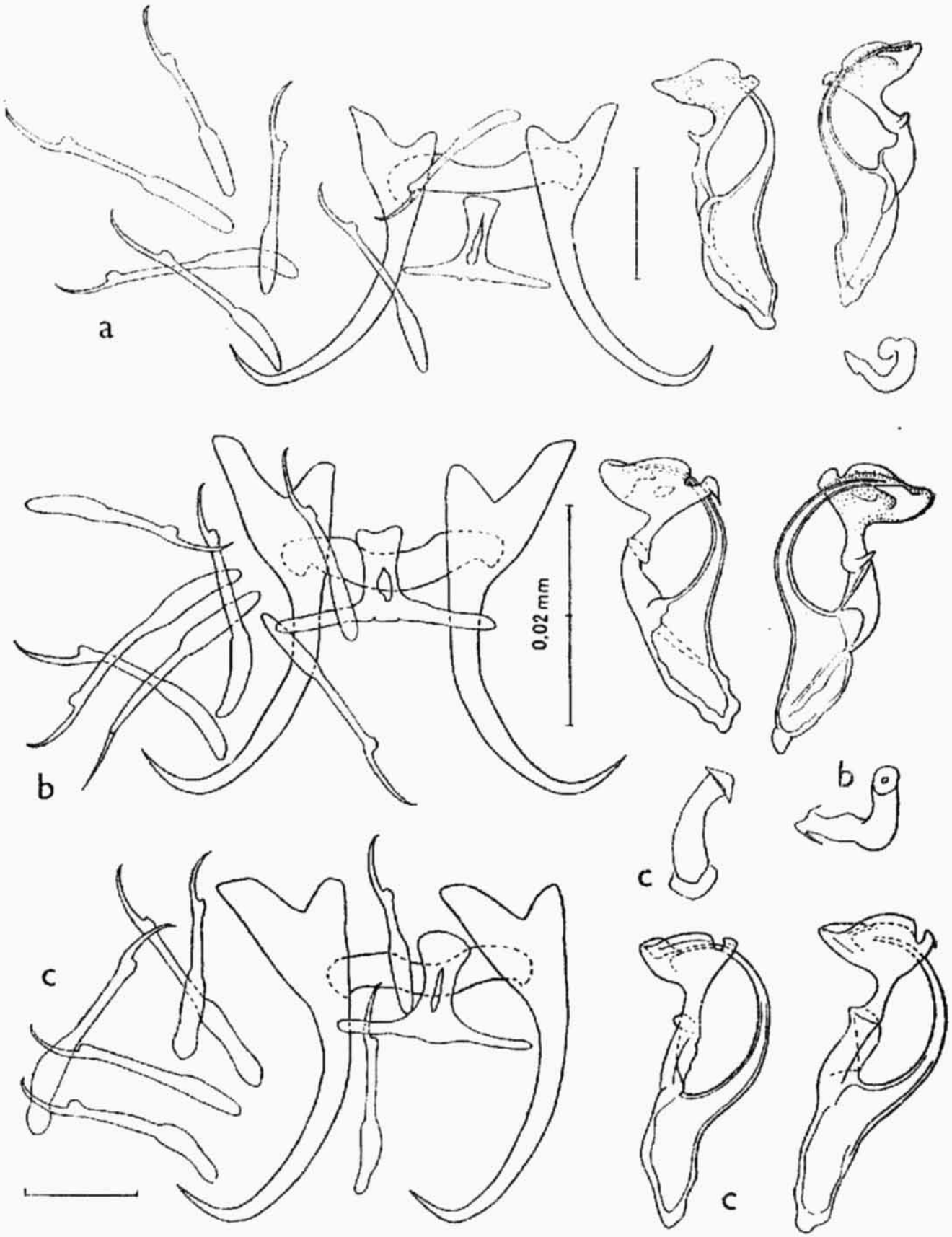

Fig. 8. *Dactylogyrus nanus* Dogiel et Bychowsky: a – from Jindřichův Hradec (the Labe river basin, Czechoslovakia), b – from the Tisa river, c – from the Iriklin damlake (the Ural river)

other fish species (*Leuciscus cephalus*, *Blicca bjoerkna* and *Abramis brama*) should be considered erroneous — apparently *D. prostae* Molnar, *D. folkmanovae* Ergens, *D. nanoides* sp. n. and *D. distinguendus* Nybelin were mistaken for it.

Dactylogyrus suecicus Nybelin, 1936 (Fig. 9)

(syn. *D. nanus* Dog. et Bych., 1934, part.)

A small worm up to 0.50 mm long, 0.12 mm wide. The haptor slightly delimited from the body by a constriction. The marginal hooks with well discernible base and shaft and a rounded projecting "opposable piece" of the point; their total length is 0.021—0.033 mm, the longest hooks being those of the 2nd and 3rd pair, the shortest of the 1st and 7th pair. The anchors with well developed roots and a small crooked point. Their total length varies between 0.032 mm and 0.041 mm, the length of the anchor base is 0.029—0.033 mm of the outer root 0.003—0.006 mm, of the inner root 0.007—0.012 mm, of the point 0.008—0.009 mm. The connecting bar is bent in the middle, has its extremities expanded backwards and measures 0.004 × 0.027—0.031 mm. The supplementary bar is ⊥-shaped, with a very stout anterior appendage which has a slit (sometimes two slits) inside, widening to end, and bifurcating into two horns or fingers; it measures 0.013—0.018 × 0.021 to 0.026 mm.

The copulatory organ consists of a tube and a supporting piece. The tube tapers to end, is sickle-shaped, with a stout, widened and extended initial part, sometimes in the form of an extended rectangular. The supporting piece starts from the anterior margin of the initial part as a ribbon-like formation which, after a constriction, expands into a saucer-shaped ending with an upturned edge, between which and the opposite margin the tube is sliding. The total length of the copulatory organ ranges from 0.025—0.032 mm, the length of the tube along the bend is 0.028—0.035 mm, the diameter in the middle 0.0015—0.002 mm, the initial part measures 0.0011—0.017 × 0.004—0.007 mm. The length of the supporting piece is 0.014—0.016 mm. The vaginal tube is in the shape of a thickened formation, often bent, with expanded or cap-like end; its length is 0.015—0.020 mm, the diameter 0.004—0.005 mm.

Location, host, distribution: gill filaments of *Rutilus rutilus*; is to be found wherever the host occurs.

The material investigated was collected from the water reservoirs of Czechoslovakia, from the rivers Tisa, Volga, Nura and the Aral Sea.

No difference was previously made between this species and *D. nanus*. However, it distinctly differs from the latter in large measurements of the body and in the chitinoid haptoral formations, particularly in the longer inner root of anchors, in the form of the anterior appendage of the supplementary bar (shaped as a vase), in the form and thickness of the copulation tube, in the structural details and stoutness of the terminal section of the supporting part, and also in bigger vaginal

305 tube. For the first time after NYBELIN, 1936, the occurrence of this species is reported by ALABOTU, 1944 and ERGENS, 1959 b.

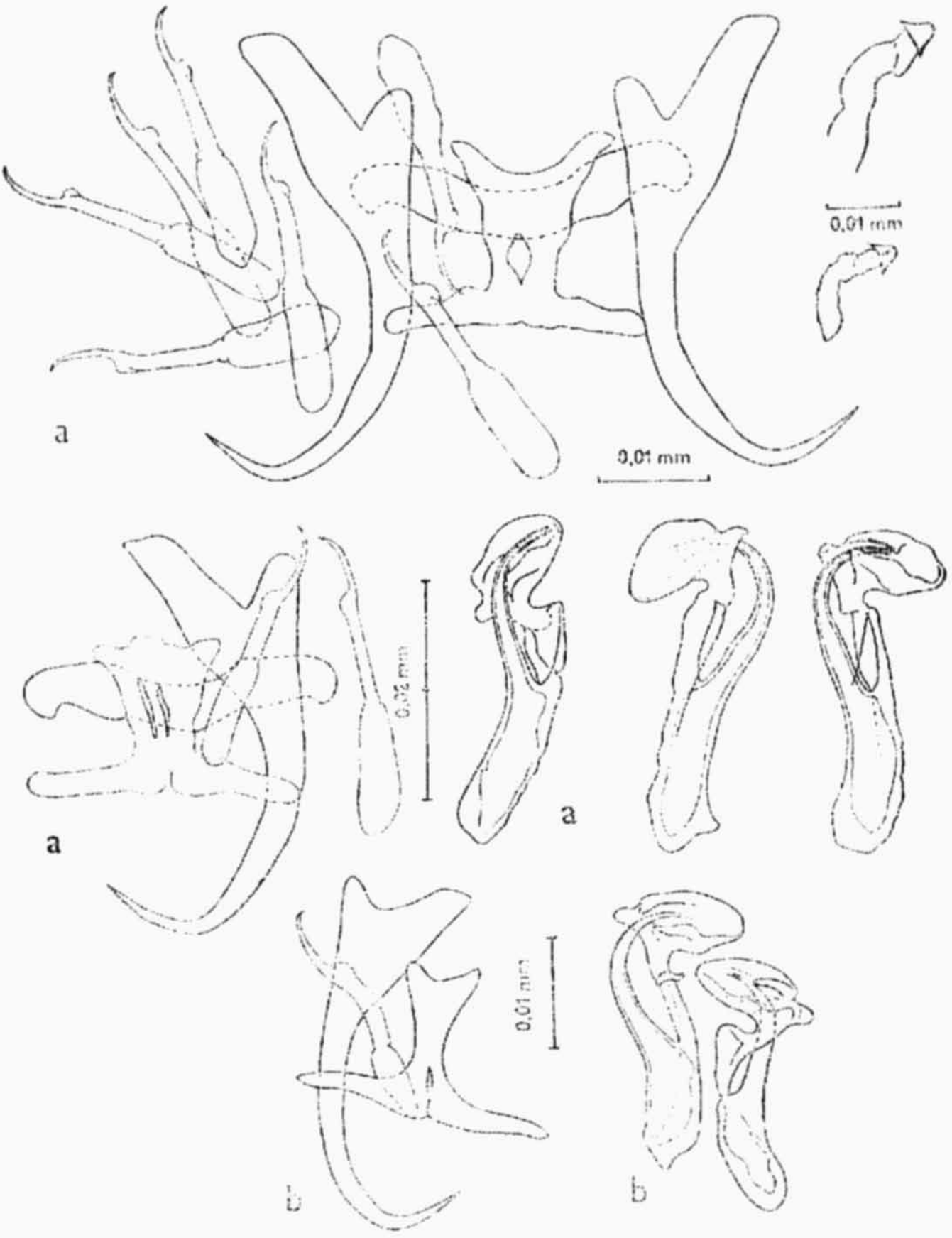

Fig. 9. *Dactylogyrus suecicus* Nybelin: a—from the Tisa river, b—from the Volga river

Dactylogyrus nanoides n. sp. (Fig. 10)

A small worm up to 0.3 mm long, 0.07 mm wide. The haptor slightly delimited from the body by a constriction. The marginal hooks are relatively thin, with well defined base and shaft, with a projecting "opposable piece" of the point; their length is 0.014—0.025 mm. The anchors with gradually curved point and well developed roots. Their total length is 0.025—0.029 mm, the length of the anchor base is 0.023—0.025 mm, of the outer root 0.003—0.004 mm, of the inner root 0.007—0.009 mm, of the point 0.008—0.009 mm. The connecting bar is narrowed and slightly bent backwards in the middle, with somewhat expanded, posteriorly pointed ends; it measures 0.002—0.003 × 0.017—0.020 mm. The supplementary bar is ⊥-shaped, with a relatively broad, somewhat widening onwards or equally thick anterior appendage and with more slender transverse appendages; it measures 0.007—0.009 × 0.013—0.016 mm.

The copulatory organ consists of a tube and a supporting piece and its total length varies between 0.020 mm and 0.022 mm. The thin tube is curved in the form of a sickle, with greatly expanded initial part which measures 0.004—0.006 × 0.008—0.009 mm, the diameter of the central part of tube about 0.0005 mm. The supporting piece in the form of a rod starting from the initial part of tube, which expands in the middle and takes the shape of a saucer, with finger-like thickenings and an appendage at its end; the supporting piece is 0.011—0.013 mm long. The vaginal armature resembles a short mushroom, measuring 0.007 to 0.008 × 0.002 mm.

Location, host, locality: gill filaments of *Leuciscus cephalus*; the Tisa river.

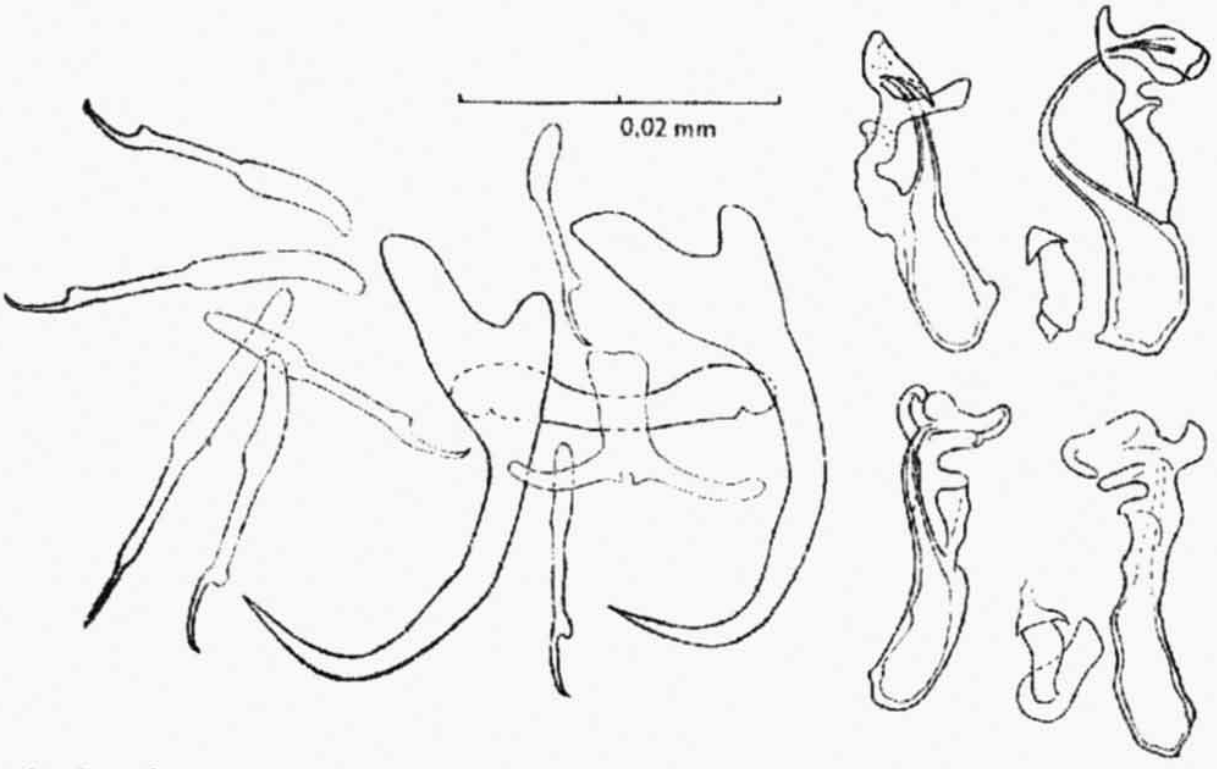

Fig. 10. *Dactylogyrus nanoides* n.sp.

This species was probably mistaken for *D. nanus*. However, it distinctly differs from the latter in the form of anchors, in greater length of their point and in the structural details of the copulatory organ.

Dactylogyrus distinguendus Nybelin, 1936 (Figs. 11, 12)

(Syn. *D. nanus* Dogiel et Bychowsky, 1934, part: *D. cornu* Linstow, 1878, part.)

A small worm up to 0.50 mm long, 0.12 mm wide. The haptor delimited from the body by a slight constriction. The marginal hooks with well defined base and shaft and with a projecting, "opposable piece" of the point which is slightly bent backwards. Their length is 0.015—0.028 mm, the longest hooks are those of the 2nd and 3rd pair, the shortest — of the 7th pair. The anchors with well developed roots and a curved small point; their total length is 0.027—0.038 mm, the length of the anchor base 0.024—0.032 mm, of the outer root 0.0025—0.005 mm, of the inner root 0.005—0.010 mm, of the point 0.006—0.009 mm. The connecting bar is bent in its central, thinner part, with its extremities expanded and sharply curved backwards and measures 0.002—0.005 × 0.020—0.028 mm. The supplementary bar is ⊥-shaped, or +-shaped, with a short posterior appendage, sometimes divided into 2 or 3 pieces; this appendage usually has a rough, indented posterior margin; the anterior appendage with a slightly expanded, convex or concave edge, has a slit (or two slits) at the base. The bar measures 0.012—0.015 × 0.017—0.022 mm.

The copulatory organ consists of a tube and a supporting piece. Its total length

Table 2. Correlation of measurements of basic formations and body length of *D. distinguendus* from various fish species (in mm)

Formation	From the fry of *Abramis brama*	From the fry of *Blicca bjoerkna*	From adult *Blicca bjoerkna*	From adult *Vimba vimba*
Anchors				
total length	0.027—0.031	0.027—0.029	0.031—0.037	0.031—0.038
length of base	0.024—0.027	0.024—0.025	0.026—0.032	0.026—0.032
length of outer root	0.0025—0.004	0.004—0.005	0.003—0.005	0.003—0.004
length of inner root	0.005—0.007	0.007	0.008—0.010	0.008—0.009
length of point	0.006—0.008	0.007—0.008	0.007—0.009	0.007—0.009
Connecting bar				
length	0.003—0.004	0.003—0.004	0.002—0.004	0.003—0.005
width	0.020—0.021	0.020—0.022	0.022—0.028	0.020—0.028
Supplementary bar				
length	0.012—0.015	0.013—0.015	0.014—0.015	0.011—0.015
width	0.018—0.020	0.017	0.018—0.021	0.017—0.022
Marginal hooks	0.015—0.022	0.015—0.020	0.018—0.028	0.016—0.027
Length of body	0.16—0.20	—	0.33—0.48	—

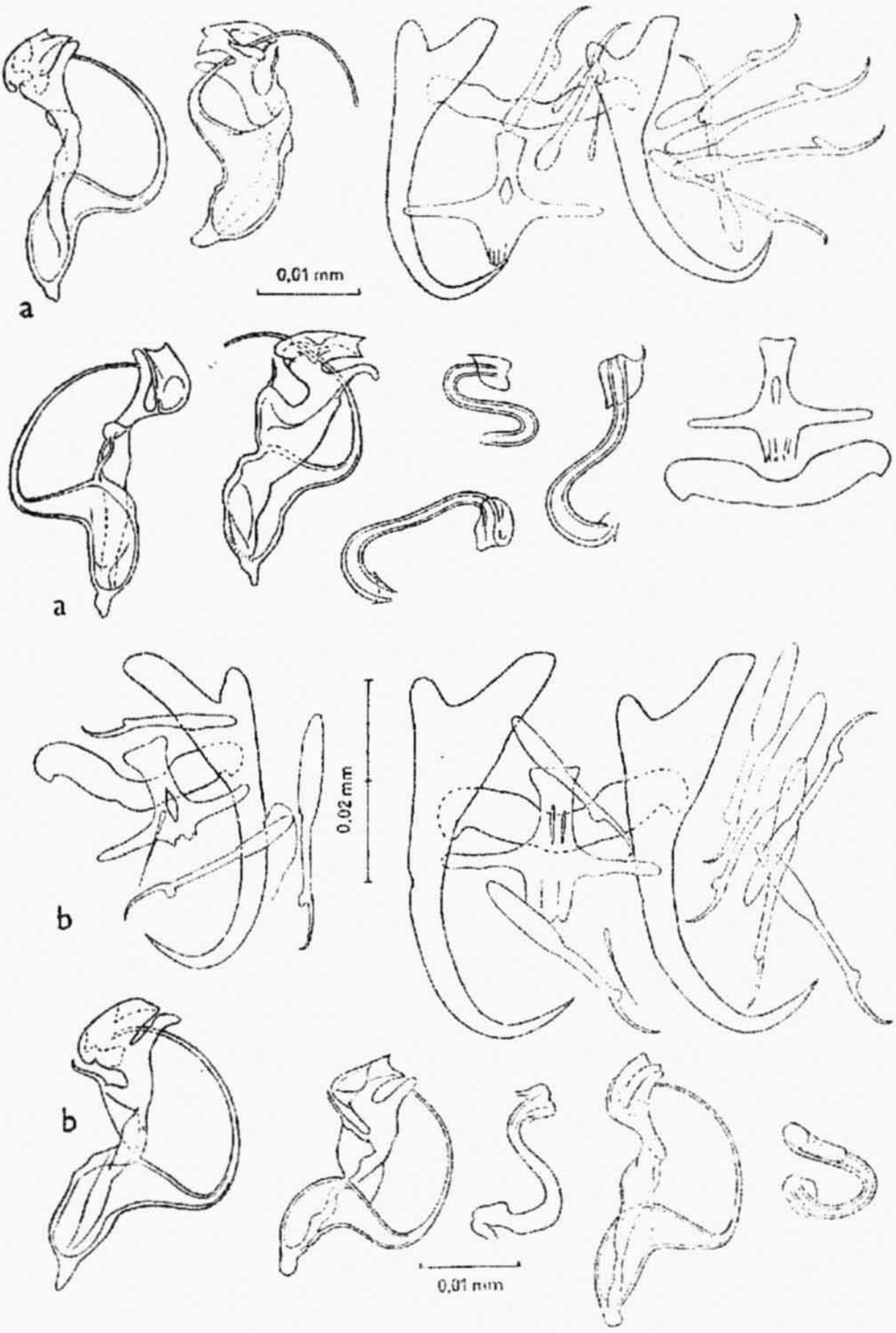

Fig. 11. *Dactylogyrus distinguendus* Nybelin: a—from the fry of *Abramis brama*, the Tisa river, Seliger Lake and Iriklin damlake, b—from *Vimba vimba*, the Tisa river

309 ranges from 0.023—0.030 mm. The tube is sickle-shaped, with the handle represented by an expanded egg-like base and the blade represented by the tapering, very thin tube proper. The initial part has an outgrowth at the back and at one side of it a thickening extends onward continuing later as the supporting piece. The latter is represented by a wide ribbon-shaped plate with a thickened margin, which becomes narrower first and then expands again at the end into a part having a finger-like appendage and a characteristic sculpture and indentation of margin. The length of the tube along the bend is 0.035—0.045 mm, the initial part measures 0.010—0.013 × 0.005—0.007 mm, the middle section is less than 0.001 mm. The length of the supporting piece (from the anterior margin of the initial part of tube) is 0.012—0.018 mm. The vaginal armature is shaped like a tube with widened ends and is usually sharply bent almost in the right angle or curved in the form of an S or a ring. Its length is 0.025—0.030 mm, the diameter is 0.0015—0.002 (in the middle section) and 0.003 mm (at the ends).

Location, hosts, distribution: gill filaments of *Blicca bjoerkna*, *Abramis brama* (fry and parr younger than one year), *Vimba vimba;* is found apparently wherever its hosts occur.

The material investigated comes from the river Tisa, the Volga delta, the Iriklin damlake (the Ural river, by courtesy of V. V. Kashkovsky) and from the Seliger Lake (by courtesy of I. V. Kulemina).

This species was previously mistaken for *D. nanus*, from which it differs in a number of features, or for a developmental stage of *D. cornu*. However, the form and measurements of its chitinoid formations and the fact that mature eggs were found in some specimens refute the identity with the latter species.

The description of the above species has been written according to all material available. However, it must be differentiated in the following way: during the investigations on the Tisa river three mature specimens of *D. distinguendus* were found in some young fishes *Abramis brama* and *Blicca bjoerkna*, caught in the ditches. The trematodes were 0.20—0.25 mm long, with a developed copulative organ, but with smaller elements of the haptoral armature than in specimens found on adult *Blicca bjoerkna* and *Vimba vimba*. Analogical observations were made during the special investigations of the fish fry carried out by Kashkovsky, Kulemina and Gläser, 1965.

Kashkovsky reported that he found the form with small elements of the haptoral armature only on the young fish *Abramis brama* and *Blicca bjoerkna*, this species being the first of *Dactylogyrus* found even on the fish fry 18 mm long. However, this species was not discovered on adult *Abramis brama*. The results obtained on the river Tisa were the same. A form with large chitinoid formations often occurs on adult *Blicca bjoerkna* and *Vimba vimba* from the Tisa river (Tab. 2). These interesting facts allow us to draw the following preliminary conclusions: 1. some species of *Dactylogyrus* parasitize only the young fish (probably those fish species which are secondary hosts?); 2. the size of the attaching chitinoid formations and

the body of some *Dactylogyrus* species depend directly upon the size (age) of the host.

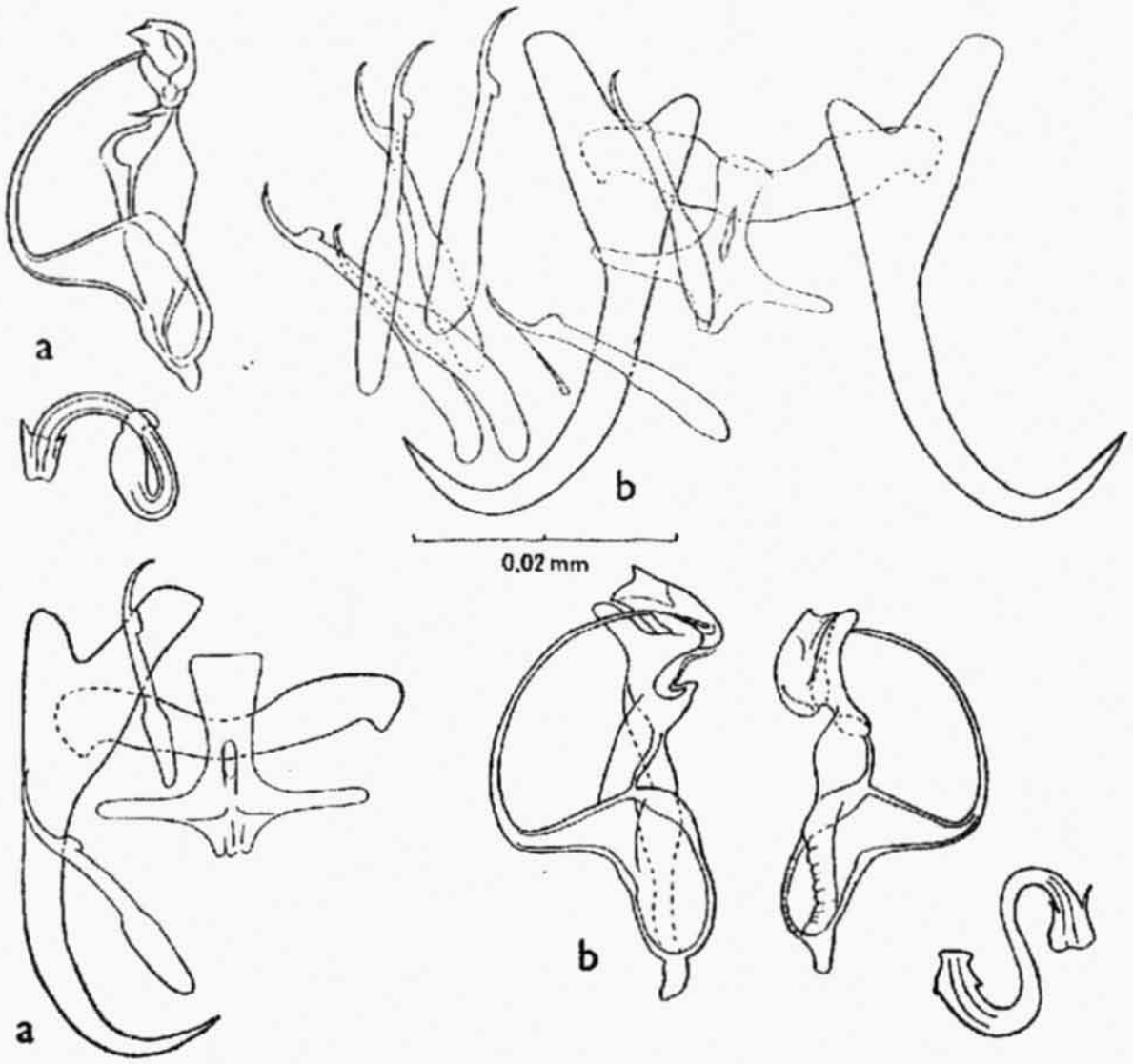

Fig. 12. *Dactylogyrus distinguendus* Nybelin: a – from the Volga river, b – from the Iriklin damlake; both from *Blicca bjoerkna*

Dactylogyrus rutili Gläser, 1965 (Fig. 13)

(Syn. *D. nanus* Dogiel et Bychowsky, 1934, part; *D. cornu* Linstow, 1878, part.)

A small worm up to 0.35 mm long, 0.08 mm wide. The haptor delimited from the body by a slight constriction. The marginal hooks with well defined base and shaft and with projecting "opposable piece" of the point. Their length is 0.017 to 0.027 mm, the longest hooks being those of the 2nd and 3rd pair, the shortest — of the 7th pair. In their form and size the anchors resemble those in *D. nanus*; their total length is 0.029—0.033 mm, the length of the anchor base 0.026 to 0.028 mm, of the outer root 0.004 mm, of the inner root 0.006—0.008 mm, of the point 0.006—0.007 mm. The connecting bar is bent in its central part, with expanded ends pointed backwards and measures 0.002—0.004 × 0.018—0.020 mm. The

311 supplementary bar is +-shaped, with a short posterior appendage characterized by a roughly outlined posterior margin and consisting of 2—3 longitudinal lobules. The anterior appendage is wide, slightly narrowed at the base (goblet-shaped) with an oval slit. The bar measures 0.014—0.016 × 0.018—0.020 mm.

The copulatory organ consists of a tube and a supporting piece. The tube is sickle-shaped, with the handle represented by an oval initial part and the blade

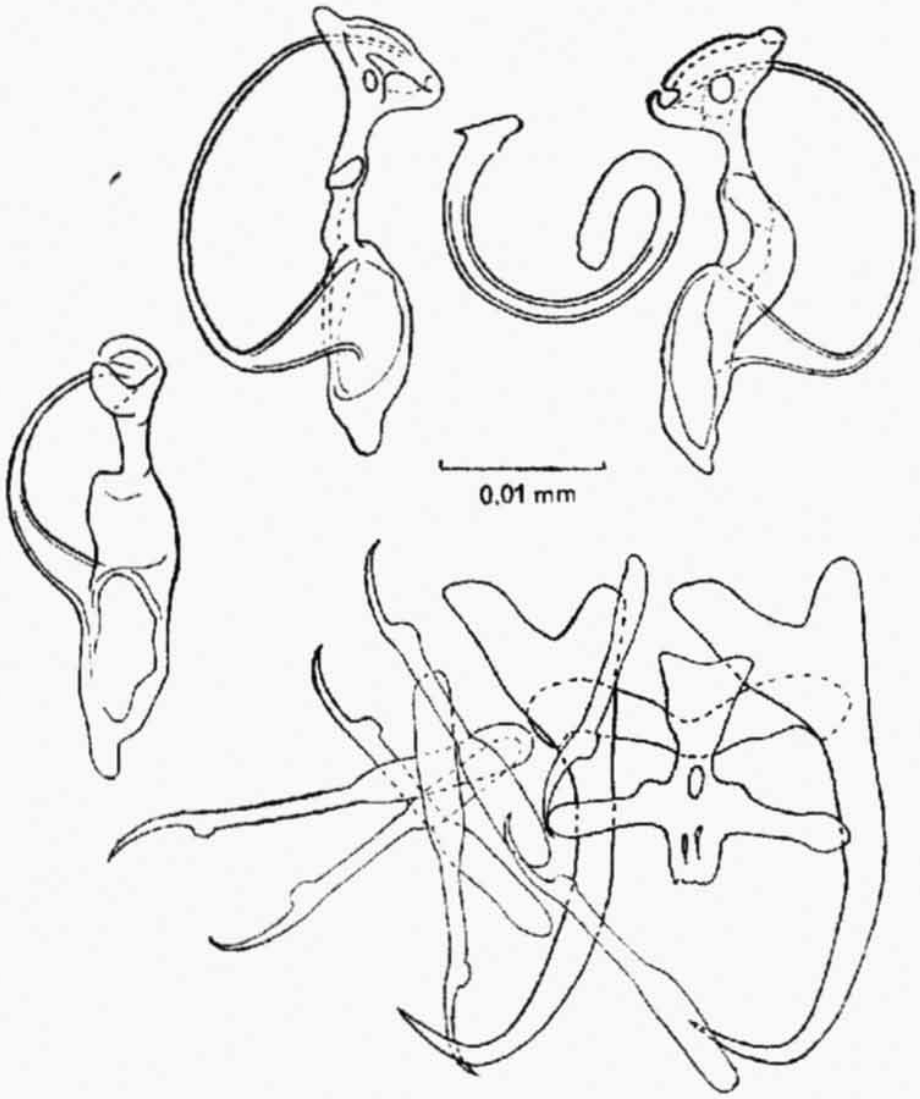

Fig. 13. *Dactylogyrus rutili* Gläser from the Tisa river

represented by the tube proper arching perpendicularly to the longitudinal axis and tapering at end. The supporting piece starts from the anterior edge of the initial section, continues as a long thickening, forms a wide plate at first and then tapers at end finally expanding into a triangular spade-like shape with a characteristic sculpture of thickened edges and a small opening in the middle. The length of the copulatory organ is 0.026—0.030 mm. The length of tube along the bend is about 0.045 mm, the initial part measures 0.010—0.012 × 0.005—0.006 mm, the diameter in the middle less than 0.001 mm. The length of the supporting piece ranges from 0.015—0.017 mm. The vaginal armature is shaped like a tube coiled

in a ring or a loop with slightly expanded ends: its length is 0.036—0.041 mm, its diameter 0.0015—0.002 mm.

Location, host, distribution: gill filaments of *Rutilus rutilus:* is found apparently wherever its host occurs.

The investigated material comes from the German Democratic Republic (by courtesy of Dr. G. I. Gläser), from the Tisa and Volga rivers.

This species was often mistaken for *D. nanus* and *D. cornu* and probably showed little difference with *D. distinguendus*. Although it belongs to the same morphological group with *D. nanus*, *D. suecicus* and *D. distinguendus*, it clearly differs from all of them in the details of the structure of its chitinoid formations, in their size and form: it differs from the first two species in its supplementary bar, the vaginal tube, in the tube form of cirrus and its structure, from the third species — in the form of the anterior appendage of the supplementary bar, in longer vaginal tube and in structural details of the terminal section of the supporting piece of cirrus. Mature eggs found in several specimens testify that this is not a developmental stage of some other species.

Dactylogyrus micracanthus Nybelin, 1936 (Fig. 14a)

(Syn. *D. caecus* Kulakowskaja, in Gussev, 1962)

This worm reaches quite a large size, being up to 1.9 mm long and 0.44 mm wide. It posseses eyes which are well perceptible in smaller specimens, but hardly visible as four little pigment conglomerations in large specimens. The haptor is very small in comparison with the stout body, is delimited from it by a constriction, and measures about 0.1 × 0.15 mm.

All chitinoid formations in comparison with the size of the body are very small (Table 3). The marginal hooks with a small base and a long shaft and a rounded projecting "opposable piece" of the point; the shortest hooks are those of the 7th pair, the longest — those of the 3rd and 6th pair. The anchors are the same type as those of *D. wunderi* Bych., with a curved point and well developed roots, of which the outer root is four times smaller than the inner one. The connecting bar is bent in its central thinner part, with expanded extremities pointed backwards. The supplementary bar is ⊥-shaped, with nearly equally thin branches.

The copulatory organ consists of a tube and a supporting piece. The tube is thin, slightly curved, with an expanded triangular initial part. The supporting piece starts from the initial part of tube as an expanded plate, continues in the form of a thin shaft-like part which widens and forks into two appendages: one is like a tongue directed onwards, the other is characterized by the expanded end which is curved backwards. The vaginal armature has the shape of an egg-like formation, with one end in the form of the convex margin of a cup.

Location, hosts, localities: gill filaments of *Rutilus rutilus*, *Leuciscus cephalus*. Water reservoirs of Sweden, Finland, the Dnestr and Tisa rivers. It was

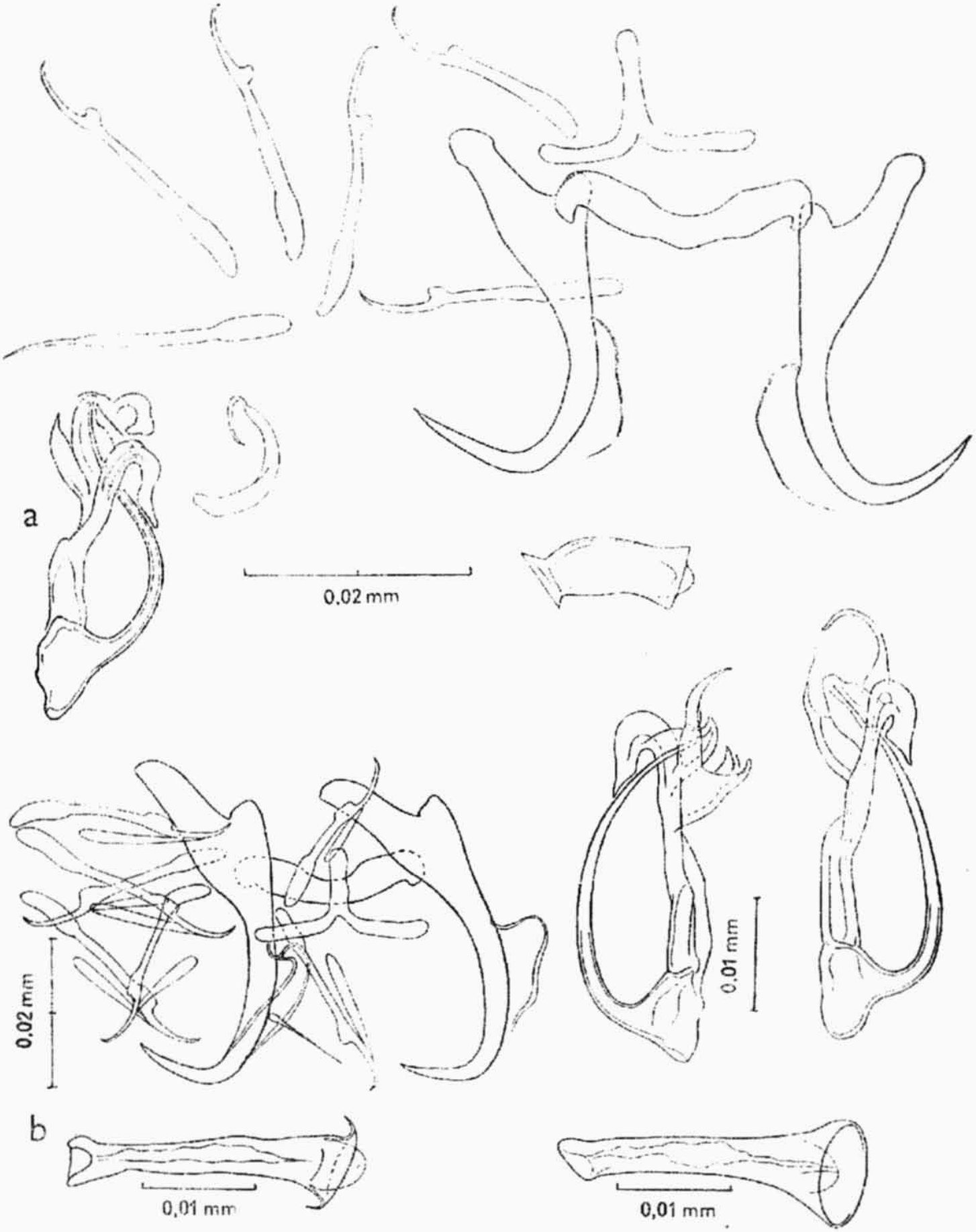

Fig. 14. *Dactylogyrus micracanthus* Nybelin from the Dnestr river (a) and *D. ramulosus* Malewitzkaja from the Iriklin damlake (b)

also found on both hosts from the Iriklin damlake (the Ural river) by V. V. Kashkovsky.

Dactylogyrus ramulosus Malewitzkaja, 1941 (Fig. 14b)

A very large worm up to 2.0 mm long, 0.4 mm wide. It posseses eyes, which are very small (in larger specimens). The marginal hooks with a small expanded (sometimes pear-like) base well delimited from the shaft and with a flattened projecting "opposable piece" of the point; the smallest hooks are those of the 7th pair, the biggest those of the 2nd and 3rd pair. The anchors (of the type *D. wunderi* Bach.), the connecting and supplementary bars and also the copulatory organ are nearly identical with those in *D. micracanthus*, differring only in measu-

Table 3. Measurements of chitinoid formations of *D. micracanthus* and *D. ramulosus* (in mm)

Formation	*D. micracanthus*	*D. ramulosus*	
		After Malevitskaya	Our data
Marginal hooks			
total length	0.016—0.026	0.019—0.022	0.021—0.032
length of point	0.006	—	0.008
total length	0.025—0.033	0.035—0.039	0.041—0.045
length of base	0.022—0.025	0.026—0.029	0.034—0.037
length of outer root	0.002—0.003	0.003—0.004	0.003—0.004
length of inner root	0.009—0.011	0.009—0.010	0.010—0.011
length of point	0.009—0.011	0.009—0.013	0.010—0.011
Anchors			
length	0.003—0.004		0.005
width	0.017—0.025	0.026—0.029	0.022—0.025
Connecting bar			
length	0.010—0.011	0.009—0.013	0.010—0.011
with	0.018—0.020		0.020—0.023
Supplementary bar			
total length	0.026—0.030	0.035—0.039	0.032—0.039
length of tube	0.025—0.030	—	0.030—0.035
size of initial part of tube	0.005—0.007 × 0.009—0.010		0.005—0.006 × 0.007—0.009
diameter of tube in central part	about 0.001		about 0.001
Copulatory organ			
length of supporting piece	about 0.020		0.025—0.029
Vaginal tube			
length	0.011—0.015	0.029—0.035	0.025—0.028
diameter	0.005—0.006	—	0.007—0.010

315 rements (Table 3). The vaginal armature in the shape of a tube expanded at its ends, one end being the double size of the other; in its general form it resembles a signal bugle.

Location, hosts, distribution: gill filaments of *Leuciscus idus* (MARKEVICH, 1951 also reports *Rutilus rutilus*).

Dactylogyrus micracanthus and *D. ramulosus* are undoubtedly closely related species and were it not for the differences between their vaginal tubes (and may be also between their marginal hooks) they could hardly be recognized as distinct species (as for example the forms of *D. ergensi*).

Considerable differences in the sizes of anchors (of their total length and the base), observed in the author's material, are somewhat alleviated by the data of MALEVITSKAYA, 1941 (Table 3), which are very dissimilar to his. It is difficult to find the cause of such discrepancy between these data. It is possible that MALEVITSKAYA found both species. As the distinction between them has been made only recently, the author proposes, before the amplitude of their features is revised on the basis of large material, to change the data concerning some measurements in the previous diagnosis of *D. ramulosus* by bringing them in conformity with his data given in Table 3.

Dactylogyrus finitimus n. sp. (Fig. 15)

(syn.: *D. cryptomeres* Bychowsky in ROMAN, 1956)

A worm of medium size up to 0.6 mm long, 0.15 mm wide. It possesses two pairs of well developed eyes. The haptor is delimited from the body by a slight constriction. The marginal hooks of the 1st and 5th pair have an elongated base well demarcated from the shaft, the hooks of the 6th and 7th pair look stouter because their shaft is wide; the "opposable piece" of the point of all hooks is flat and hardly projecting. The total length of hooks ranges from 0.026—0.033 mm, the shortest hooks being those of the 1st, 4th and 5th pair, the longest — of the 6th pair. The anchors are massive, with curved point, of the *D. wunderi* Bych. type with a well developed inner root and a small outer root. Their total length ranges from 0.040 mm—0.045 mm, the length of the base is 0.033—0.038 mm, of the outer root 0.002—0.005 mm, of the inner root 0.010—0.014 mm, of the point 0.010 mm. The connecting bar is slightly bent backward both in its central part and in its extremities, which are curved backward like hooks; it measures 0.006 to 0.008 × 0.026—0.030 mm. The supplementary bar is V-shaped, with lateral ends always directed onwards and with a thickened middle part from which several appendages of various size and form extend, sometimes giving the bar the semblance of a cross; the bar measures 0.010—0.015 × 0.021—0.025 mm.

The copulatory organ consists of a tube and a supporting piece. Its total length is 0.040—0.042 mm. The tube is sickle-shaped in its proximal part, almost straight in its distal part, slightly expanded in its initial part, then almost cylindrical; the

initial part is surrounded by a chitinoid thickening which often forms a small triagular appendage; the tube is terminated by a tongue-shaped expansion with an oval opening. The length of tube along the bend is 0.052—0.058 mm, the diameter of the initial part 0.005—0.006 mm, of the middle part about 0.003 mm. The supporting piece starts from the initial part of tube as an elastic band intersecting the bend of tube and continuing as groove-like part which envelops the tube. One side of the groove forms a powerful claw supporting the tube, along which a membrane (or ligament?) is often visible. The vaginal armature was not detected.

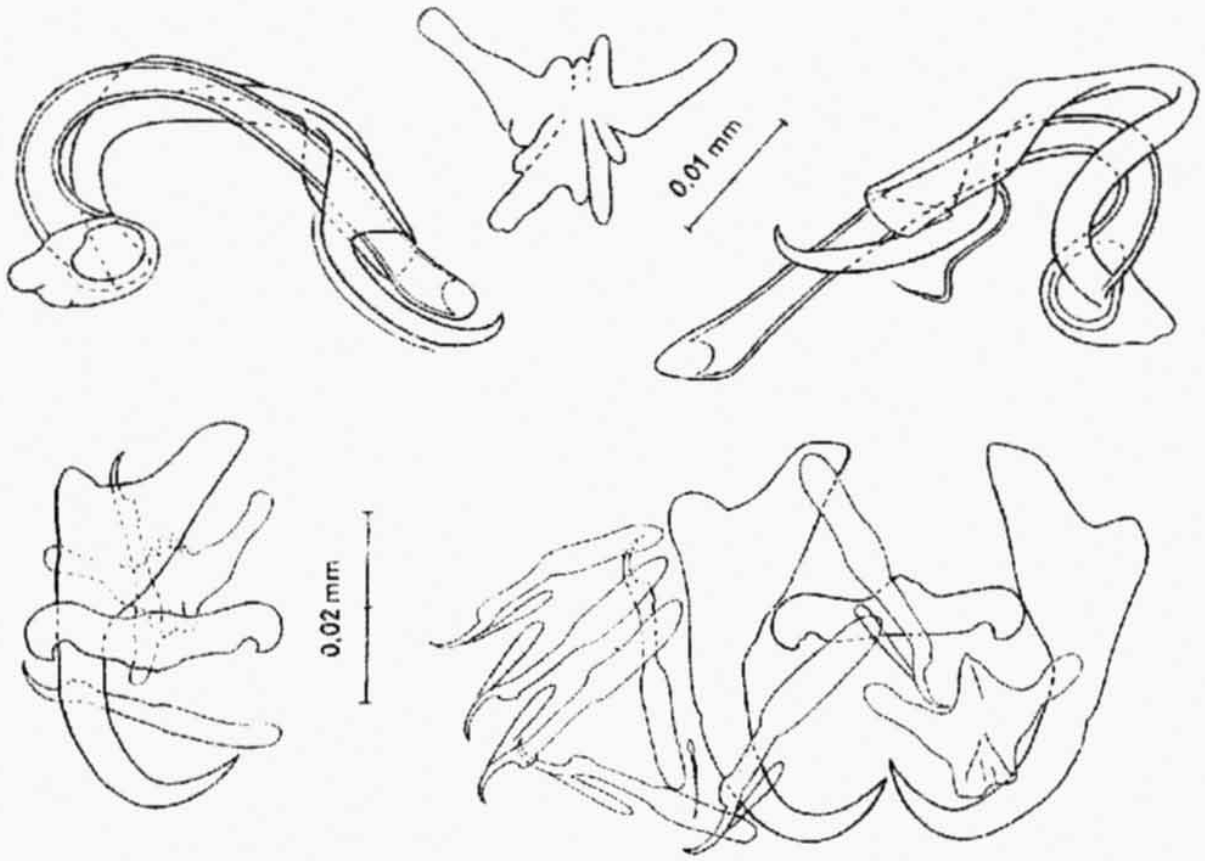

Fig. 15. *Dactylogyrus finitimus* n.sp.

Location, host, locality: gill filaments of *Gobio gobio;* the Latorica river near the town Čop.

15 specimens were investigated.

Dactylogyrus finitimus belongs to the "cryptomeres" group, which includes closely related species *D. cryptomeres* Bychowsky, *D. gobii* Gvosdev, *D. gobioninum* Gussev and *D. pseudogobii* Achmerow. It differs from them all in massive anchors, in the form of the supplementary bar and in the characteristic terminal expansion of tube of the copulatory organ (in other species the tube is merely obliquely cut).

Dactylogyrus haplogonoides n. sp. (Fig. 16)

A worm of small or medium size up to 0.6 mm long, 0.15 mm wide. The haptor delimited from the body by a slight constriction. The marginal hooks with well

317 demarcated base and shaft, with slightly projecting flattened "opposable piece" of the point; their length ranges between 0.026—0.040 mm, the longest hooks being those of the 2nd and 3rd pair, the shortest — of the 7th pair. The anchors with well developed roots, with a slightly curved sabre-shaped point; their total length is 0.042—0.051 mm, the length of the base 0.036—0.043 mm, of the outer root 0.003—0.006 mm, of the inner root 0.010—0.014 mm, of the point 0.009 to 0.011 mm. The connecting bar is bent backward in its thin central part, its extremities are slightly expanded and pointed posteriorly; it measures 0.004—0.007 × × 0.030—0.040 mm. The supplementary bar is cross-shaped or ⊥-shaped, with a hardly visible intented posterior appendage which is always shorter than the anterior one; the bar measures 0.018—0.22 × 0.024—0.031 mm.

The copulatory organ consists of a tube and a supporting piece. Its total length is 0.070—0.105 mm. The tube is fairly wide, tapering to end, spirally coiled, forms 2—2.5 coils, of which the terminal one is expanded; its initial part is widened, elongated, with a constriction and opening in the middle; the posterior margin of the initial part with a massive, nearly straight appendage which is slightly expanded backwards; the length of tube along its bend (without appendage) is 0.120—0.135 mm, the diameter of the middle part about 0.002 mm, the initial part measures (without appendage) 0.023—0.028 × 0.010—0.15 mm, the length of appendage 0.003—0.015 mm. The tube is surrounded with a thin membrane-like case. The supporting piece is like a band starting from the initial part of tube, its end is expanded in the form of a pear with a small claw at the end or with a blade on one side. The vaginal armature was not detected.

Location, host, locality: gill filaments of *Vimba vimba;* the rivers Tisa, Don (after oral communication of N. I. Krasil'nikova).

This species is closely related to *D. haplogonus* Bychowsky, described from the Caspian region and found in some rivers of the Caspian basin, differing from it in the considerably longer and tapering tube and in its more massive initial part. Apparently these two species originate from one common ancestor which once inhabited the ancient Ponto—Aralo—Caspian basin. After this basin had broken down into the Black Sea basin the said ancestor produced *D. haplagonoides* in this region and *D. haplagonus* in the Caspian region.

At present information has accumulated on the presence of dimorphism and polymorphism in some groups of *Dactylogyridae*. Besides the mentioned variability of different populations of *D. ergensi*, and also of the described "fry" form of *D. distinguendus*, the author has obtained data on the dimorphism of *D. crucifer*, *D. linstowi*, *D. zandti* and some others. In the case of *D. zandti* the same phenomenon may be observed as in the *D. distinguendus:* the fry of *Abramis brama* are parasitized by a form with chitinoid haptoral formations twice as small as those in the form parasitizing the adult fishes, while the copulatory organ and the vaginal tube are nearly of the same size in both forms. The variability of *D. crucifer* and *D. linstowi* is manifested by another phenomenon: as in *D. ergensi* the chitinoid formations become smaller in some populations. For example the populations of *D. crucifer*

from a number of water reservoirs in Central Asia (Kairak-kum water reservoir, rivers Uzen, Sary-su) possess much smaller copulatory organ and haptoral armature than the parasites from the rivers Ural, Tisa and others. Both forms sometimes occur together (for example in the river Ural both the "large" form and the "small" form were found by V. V. Kashkovsky, but the latter occurred in smaller numbers). However, in order to understand the causes of such dimorphism they cannot be

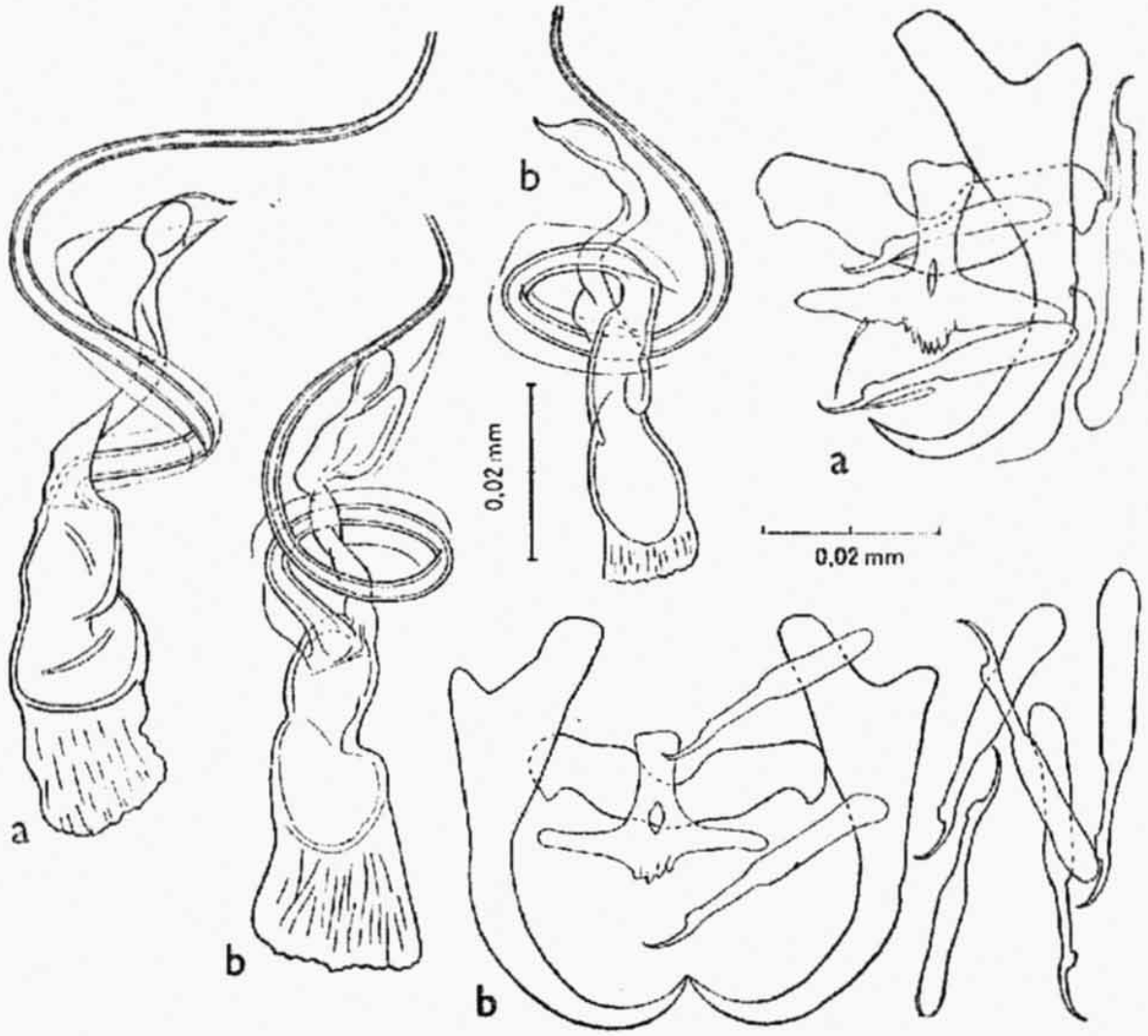

Fig. 16. *Dactylogyrus haplogonoides* n.sp.: a—from the Bečva river (the Morava river basin, Czechoslovakia), b—from the Tisa river.

studied during occasional observations, but must be traced during various seasons and in various age groups of fishes in each region where the experiments are carried out, where parasite monocultures are cultivated and conditions for hybridization of various species are formed etc.

Apart from dimorphism within the framework of some species, there are many examples of it side by side on identical hosts of related species. Such "pairs" are *D. nanus* Dogiel et Bychowsky and *D. suecicus* Nybelin, *D. crucifer* Wagener and *D. caballeroi* Prost from *Rutilus rutilus; D. cryptomeres* Bychowsky and *D. finitimus* sp. n. from *Gobio gobio; D. cornu* Linstow and *D. cornoides* Gläser et Gussev from

319 *Blicca bjoerkna; D. difformis* Wagner, *D. difformoides* Gläser et Gussev and *D. izjumovae* sp. n. from *Scardinius erythrophthalmus; D. amphiothrium* Wagener and *D. hemiamphibothrium* Ergens from *Acerina cenua*, *D. macracanthus* Wagener and *D. tincae* Gussev from *Tinca tinca* and others. The groups of closely related species of *Dactylogyrus* occur on many fishes of the Far East (Gussev, 1955, 1962). A similar picture may be observed in other genera, for example *Ancylodiscoides Yamaguti* (Gussev and Strelkov, 1960).

Finally, there is often a third case when closely related parasite species occur on closely related hosts, for example *D. distinguendus* Nybelin and *D. rutili* Gläser, *D. micracanthus* Hybelin and *D. ramulosus* Malewitskaja and others.

It may be supposed that in all three phenomenons—in the presence of polymorphism both in the same and in different regions, in the differentiation of various species on one or various hosts—there are different stages and cases of divergence of specific parasites, which apparently is taking place before our eyes.

Time and again during the repeated investigations of fish parasites in some water reservoirs a considerable variability in the species composition and distribution of *Dactylogyrus* was observed by the author in different years. This fact testifies to the great sensitivity of many species to some unknown factors of environment. If they are sensitive under conditions of relative geological stabilization of environment, the great events such as were the sea transgressions and regressions in the Quaternary period must have left a mark not only in their composition, but probably also provoked an increase of their variability. The emerged forms could diverge in various ecological and geographical niches, both on the same and on various fish species, on the one hand within the framework of one water reservoir and on the other hand in various water reservoirs which had been disrupted by the sea transgressions. During the merger of rivers which followed at the time of regression the previously disrupted populations of hosts mixed again and could exchange young forms (species) of *Dactylogyrus* which emerged on them during the isolation. In this way closely related species occurring side by side on one host most probably came into being, for example *D. ergensi*, *D. dirigerus*, *D. elegantis*, *D. nanus* and *D. suecicus* etc. If the fresh-water basins of the Black and Caspian Seas were united now (for example by means of the Volga-Don canal) and the sea water ceased to prevent the fishes from wandering from one water basin into another, *D. haplagonus* and *D. haplagonoides* would probably occur together everywhere in Azov-Caspian basins on *Vimba vimba* in due course of time.

These are most probable routes by which polymorphism originated, the pairs or groups of species on the same or closely related hosts emerged (Gussev 1955, 1964). It is also most probable that some "species" may be in reality hybrid forms of other species and therefore are not entitled to exist as species. Similar suppositions, however, must be verified by experimental investigations.

1. The described 7 new species *Dactylogyrus rarissimus*, *D. izjumovae*, *D. dirigerus*, *D. elegantis*, *D. volgensis*, *D. nanoides*, *D. finitimus* and *D. haplogonoides* differ from the other known species in the structure of the copulatory organ and haptoral chitinoid formations.
2. *Dactylogyrus nanus*, *D. suecicus* and *D. rutili* occur only on *Rutilus rutilus*. The records on the findings of *D. nanus* on *Leuciscus cephalus*, *Blicca bjoerkna* and *Abramis brama* should be considered erroneous, as it was mistaken for other related species.
3. Polymorphism of some species and also the presence of groups of related species indicate the possible routes and stages in the evolution of *Dactylogyrus* species, especially under conditions of interchange of phases of the sea regression and transgression.

REFERENCES

ALAROTU H., Untersuchungen über die an Fischen in Finland lebenden monogenetischen Trematoden. Acta Zool. Fenn. 43: 1–52, 1944.

GLÄSER HANS-J., Zur Kenntnis der Gattung *Dactylogyrus* Diesing 1850 (*Monogenoidea*). Z. f. Parasitenk. 25: 459–484, 1965.

–, GUSSEV A. V., (Some errors in the systematics of the European *Dactylogyrus* species) (in press) (in Russian).

GUSSEV A. V., (Monogenetic trematodes of the fishes in the Amur river basin.) Tr. Zool. inst. AN SSSR 19: 171–398, 1955 (in Russian).

–, (Monogenetic trematodes. The key of parasites of freshwater fishes of the USSR, edited by B. E. Bykhowsky.) Izd. AN SSSR. Moskva-Leningrad 1962 (in Russian), Jerusalem 1964 (in English).

–, Analysis of the parasitofauna of fishes in the Far East. Parasit. worms and aquatic condit., Prague: 187–195, 1964.

–, (New data in the study of freshwater *Dactylogyridae*.) Symposium on parasites and fish diseases in water reservoirs (January 26–28, 1965). Tez. dokl. 8–10, 1965 (in Russian).

–, STRELKOV JU. A., (*Ancylodiscoides* (Monogenoidea) of the sheatfish (*Silurus* and *Parasilurus*) of the Far East.) Tr. Zool. inst. AN SSSR 28: 197–255, 1960 (in Russian).

ERGENS R., K problému druhové samostatnost: *Dactylogyrus nybelini* Markewitsch. 1933 (*Monogenoidea*). Věst. Českosl. Zool. Spol., Acta Soc. Zool. Bohem. 23 (2): 156–160, 1959a.

–, Nálezy dalších druhů rodu *Dactylogyrus* Diesing (Monogenoidea) v ČSR a vliv lokalisace na morfologické změny chitinových částí haptoru *Dactylogyrus hemiamphibothrium*. Čs. parasitol. 6 (1): 87–92, 1959b.

KULAKIVSKA O. P. (Parasites of fishes in the upper reaches of the Prut river.) Nauk. Zap. nauk. – prir. muzeu AN URSR 8: 70–82, 1960 (In Ukrainian).

LUCKÝ Z., Příspěvek k poznání žabrohlístů jižní Moravy. Sb. VŠ zemědělské a lesnické v Brně, ř. B. Sp. fak. veter., V (XXVI) 2: 124–157, 1957.

MALEVITSKAYA M. A., (New species of the genus *Dactylogyrus* Diesing 1850 *(Trematoda)* from the Dnepr river.) Dokl. AN SSSR 30: 267–269, 1941 (in Russian).

MARKEVICH A. P. (The parasite fauna of freshwater fishes of the Ukrainian SSR.) Izd. AN UDSSR, Kiev, 1951 (in Russian).

MIZELLE J. D., PRICE C. E., Additional haptoral hooks in the genus *Dactylogyrus*. J. Parasit. 49, 6: 1028–1029, 1963.

–, Studies on Monogenetic Trematodes. XXVIII.

Gill parasites of the Piranha with proposal of *Anacanthorus* gen. n. J. Parasit. 51 (1): 30—36, 1965.

MOLNAR K., Zwei neue monogenetische Egelarten. Acta veterinaria Acad. Sc. Hung. 14: 247—252, 1964a.

—, Über die Parasitenfauna der Fische in Ungarn. II. Bekannte und neue *Dactylogyrus*-Arten an einheimischen Fischen. Acta veterinaria Acad. Sc. Hung. 14: 455—467, 1964b.

NYBELIN O., Kleine Beiträge zur Kenntnis der Dactylogyren. Ark. f. Zool. 29A (3): 1—29, 1936.

PRICE C. E., MIZELLE J. D., Studies on monogenetic trematodes. XXVI. *Dactylogyrinae* from California with the proposal of a new genus *Pellucidhaptor*. J. Parasit. 50: 572—578, 1964.

The Advantages of Combining the Indirect Fluorescent Antibody Test and the Intradermal Test in the Diagnosis of Toxoplasmosis

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Abstract. 291 persons were examined for the presence of toxoplasmic antibodies by two methods, the intradermal test (IDT) and the indirect fluorescent antibody test (IFAT). In comparing the results it was found convenient to use a combination of both methods, performed in that way that, simultaneously with the IDT blood, extracted by finger puncture was placed on filter paper and, after drying, examined by IFAT. Positive results of this examination indicate the necessity of performing repeated quantitative examinations of the serum by IFAT. The titer level of IFAT demonstrable antibody and mainly its changes give evidence of the phase of infection. In combining the IDT with the qualitative examination of the dried blood on the filter paper it is possible to reduce the number of sera which should be further examined by the quantitative IFAT.

In recent years, Coons' immunofluorescent antibody technique has been applied more frequently for the serological diagnosis of toxoplasmosis. GOLDMAN (1957a, b, 1962) suggested the use of the fluorescent inhibition test (FIT) for these examinations, KRAMÁŘ (1961) drew attention to the advantages of the indirect fluorescent antibody test (IFAT). The successful employment of IFAT for the serological diagnosis of toxoplasmosis has also been reported by a number of other authors (DESARAM et al. 1962, KELEN et al. 1962, MANDRAS et al. 1962, BERENGO 1962, RUCKERBAUER et al. 1963, GARIN and AMBROISE—THOMAS 1963, STADTSBAEDER et al. 1964, CAMARGO 1964, VAN NUNEN and VAN DER VEEN 1965). This illustrates the increasing importance of this method for the serological diagnosis of toxoplasmosis.

As in every other new diagnostic method, also in IFAT we had to examine its relation to the reactions which are still used for the serological diagnosis of toxoplasmosis. In earlier papers (KRAMÁŘ 1962, 1964) we established the qualitative relations between IFAT, FIT and CFR, evaluating them qualitatively in our report (KRAMÁŘ et al. 1964). We also published the results of our studies on the relation of IFAT to the Sabin—Feldman reaction (KRAMÁŘ et al. 1966).

The object of this paper is an investigation of the relation of IFAT to the intradermal test (IDT). Although we are comparing two reactions of a very different

nature the results indicate that a combination of these two methods may lead to certain conclusions, which could be of importance for the clinic.

MATERIAL AND METHODS

Indirect fluorescent antibody test (IFAT)

Antigen was prepared principally after GOLDMAN (1957): toxoplasmas from the peritoneal exudate of mice, three days previously infected intraperitoneally with *Toxoplasma gondii*, strain CB, were fixed for one hour with 1% neutral formol in buffered saline; after centrifugation, the sediment was resuspended in saline to the desired consistency, transferred to slides, dried and kept at a temperature of −20 °C. For details see KRAMÁŘ et al. (1964).

Conjugates: we employed gamma globulin from rabbits and goats against the human gamma globulin, conjugated with fluorescein-iso-thiocyanate (FITC), a product of the Institute of Sera and Vaccines, Prague. Before use, we saturated them once or twice with dried pork liver powder, prepared after CHERRY et al. 1959. Before using the conjugate we titrated it in such a way as to obtain in a reaction with a negative serum and without a serum at the mentioned dilution of the serum a negative reaction and, a well marked positive reaction with the positive control serum.

The performance of IFAT: In a major part of this work the serum was replaced by eluates of human blood, placed on filter paper and dried. This method, elaborated by ANDERSON et al. (1961) for schistosomiasis, was adopted and confirmed by us for employment in the diagnosis of toxoplasmosis (KRAMÁŘ 1965). We proceeded in the following manner: the blood obtained by finger puncture with a sterile hypodermic needle was placed on circles of filter paper Whatman No. 3 (20 mm in diameter) and left to dry at room temperature to be examined within a fortnight after extraction. The circles with the dried blood were cut out, rolled up and inserted into flexible tubes, stoppered at one end with a small test-tube. The circles of filter paper containing the dried blood, were moistened with 0.2 ml of buffered saline, pH 7.2 (phosphate 0.01 M) and the tubes were placed in the moist chamber for one hour, manipulating them several times between the thumb and the forefinger. The eluate, forced from the flexible tube by inverting it in a small vise, was placed on slides covered with smears of toxoplasmas.

The human sera used in the second part of our work were diluted with buffered saline (PBS) starting from 1 : 10; the eluates of the dried blood were not further diluted. The serum, respectively the eluate of the dried blood, was allowed to act for 30 min in the moist chamber at 37°C, then washed in PBS on the mechanical shaker for 5 min. and under tap water for 5 min. and left do dry at room temperature. The titered conjugate was placed in the moist chamber for 30 min. at 37°C, then the antigen was washed and left to dry in the air in the same manner as after the action of the serum. For removing the last traces of unspecific staining, we performed a complementary staining with Evans' blue after NICHOLS et MCCOMB (1962) (1 : 5,000 in PBS, applied for 15 min. at 37°C). This was followed by washing and drying in the same way as after the action of the serum. The preparation was mounted in buffered glycerol.

Microscope: We used the Soviet microscope ML-2 with incident UV-light arrangement, excitation filter FS-1,2 mm (the same as the Zeiss filter BG_1) combined with the CZC-14, absorbing the red radiation and with the filter BC-8 against fading. As protection filter we used ŽS-18 (= Zeiss OG). The preparations were read with a binocular head, using an immersion objective X90, and an ocular X4.

Evaluation of the fluorescence:

0 = only moderately red fluorescence of the antigen stained with Evans' blue;

\+ = distinct green fluorescence, but a pale peripheral fluorescence of the toxoplasmas; the cytoplasm pale red;

\+ + = intensive green peripheral fluorescence of toxoplasmas, the cytoplasm pale red.