FOLIA PARASITOLOGICA (PRAHA) 26: 103–109, 1979.

METHODS AND TERMINOLOGY IN THE STUDY OF MONOGENEANS

A. V. GUSSEV

Zoological Institute of the Academy of Sciences of the USSR, Leningrad

Abstract. The necessity for accurate identification of species in any monogenean research and fauna sampling is emphasized. This is impossible without strictly keeping to the following requirements: high quality of preparations, photographically precise large-scale drawings and measurements according to a united scheme. The author gives some practical advice on technique and treatment of certain diagnostic criteria opposed to formal estimation of features made by some specialists. Examples of terminological confusion are given.

Classification of organisms provides a foundation for the study of all living things. Each species has its morphological and biological characteristics, its way of interacting with the environment, and its history. Only by putting together information on individual species can one get an idea about a group (genus, family, etc.), and formulate broader principles. Such is the path of inductive, empirical learning. That path is followed also by V. A. Dogiel's school of ecological parasitology. Although generalized observations often provide impetus to progress along it, they make sense only after they have been verified at the level of individual phenomena, which themselves can be qualitatively different.

Transposing this statement to the field of helminthology, one can say that it is sometimes possible to agree with information on trematodes, cestodes or monogeneans, presented in summary reviews, while rejecting data or conclusions on, for example, ancyrocephalids, a very confused group, or on dactylogyrins in general, when dealing with influences exerted upon them by environmental factors in various localities. These factors can have different (sometimes opposite) effects on different species, even on the same host. A generalized approach produces average data, not applicable to any one species and quite incapable of promoting knowledge of general problems. In the same way, obsolete information about groups with poorly known systematics cannot be uncritically used for analysis of, and conclusions on, zoogeography or ecology, for which it is sometimes used.

Therefore, any biologist and parasitologist, observer and experimenter, should know the species with which he is dealing (and be able to depend on identifications of species with which his predecessors worked). He must distinguish between species and know how to obtain material required for this purpose, such as suitable preparations, drawings and measurements. Elementary though these procedures might be in all research, they have not been mastered by some even quite experienced specialists, especially general parasitologists and helminthologists. This is particularly true with regard to the study of methodologically difficult group of small lower monogeneans. Since they constitute 30—40 % of the entire parasite fauna and up to 70 % helmith fauna of freshwater fishes, even the smallest methodological error may lead to gross inaccuracy in much of the data obtained. This author experienced several instances in which up to 90 % of specimens brought to him for verification were glycerine-gelatine preparations unsuitable for specific diagnosis. Worms so prepared were not strongly enough pressed and characteristics distinguishing between some closely related species could not be observed. Measurements of such specimens gave incorrect figures; their chitinoid structures were slanted and did not show their true dimensions. In consequence, statistical treatment of such data gives incorrect results. Our collegues sometimes waste months of their time on the collection and "processing" of such "material". All this work has to be repeated, but we do not know

whether it always is, or whether imperfect material is being used as reliable in papers and dissertations.

Poor quality of preparations is often due to impurities and contaminants occurring in water, in poorly filtered glycerine-gelatine or in the air. It might be due to the fact that an uneven coverslip is lowered with its concave side on the object. A coverslip might be too thick, or the worms might "scatter" from its margins. There are other, easily avoidable causes. To produce well stained preparations of higher or large lower monogeneans (small dactylogyrineans stain poorly), the worms must be fixed alive (or killed by heating on the slide directly before fixing), flattened by coverslip or a piece of another slide, with a weight on it, if required (Bykhovskaya 1969, Khotenovsky 1974). Good results with lower monogeneans are obtained by using ammonium picrate (Gussev 1968). This method is convenient because of its simplicity and the very short time needed to make preparations. However, such preparations deteriorate on long storage (more than a year). If needed for a collection, they should be transferred to glycerine-gelatine. The method of making preparations by means of polyvinyl-alcohol is still simpler. True such medium makes some structures too translucent but such effect can be weakened by reducing (or excluding) the portion of lactic acid in the reagent (from personal communication with Dr. V. J. Trofimenko).

It is particularly unfortunate that preparations of holotypes and paratypes, which we receive for examination from various collections, national collections of some countries included, prove to be poor in some instances. Much of what has been shown in original species descriptions, even chitinoid structures, cannot be seen. Sometimes we even receive dessicated "picrate" preparations (without protective sealing along margins of coverslips), unsuitable for study without remounting, which does not always yield satisfactory results.

In addition to the quality of preparation, the value of the material is ensured by making collections as complete as possible. Even in very intensive infections, all worms from at least half of the gill arches, fins and body surface should be collected. In practice, however, small individuals (or species) are often overlooked. The other half of the gills should be fixed in 4 % formalin, so that worms can be collected from them later. When examining the fish for monogeneans, one must not forget to check the urinary bladder and ducts, nasal cavities and intestine.

It is absolutely necessary for all those who work with monogeneans, as indeed with other groups, to develop a habit of making photographically accurate, large-scale drawings of chitinoid and other structures, important in species diagnosis (with use of drawing apparatus and appropriate phase contrast optics). It is also important to develop techniques for microphotography. A photograph is the most objective document, but the focal depth of objectives is shallow and not all details come out sharply. On the other hand, photographs, in addition to required details, record many superfluous ones. Therefore, they cannot fully replace drawings. Series of drawings of each species are needed, not so much for publication as for development by the investigator himself of the ability to distinguish species at first sight. The human brain and eye usually take appropriate notice only of that which the pencil has recorded. Time and effort spent on developing drawing techniques pay handsome dividends later. Drawings similar to those in old publications on *Gyrodactylus*, reproduced in "Key to Parasites of Freshwater Fishes of the USSR" (Gussev 1962), are no longer acceptable. Unfortunately, similar and even poorer illustrations are sometimes received by editors with papers and slip through to publications. All chitinoid structures of the haptor should be measured and illustrated, with scale lines, only in profile, with all angles and curves adequately displayed. Sometimes only these details allow the identity of the species to be determined (Gussev 1967). Therefore, in drawings of *Gyrodactylus* a marginal hook is mandatory; for *Dactylogyrus* and all those in which hooks of different pairs differ from one another, one half of the set of hooks should be illustrated. The copulatory organ should be drawn in two or three positions, showing the volume of its structures. To make accurate drawings of hooks and anchors in higher monogeneans, in mono-

cotylids, capsalids, etc., separate preparations must be made from dissected and squashed haptor.

Publications of descriptions and drawings should be permissible only when they are better, fuller and more accurate than the original descriptions. With the catastrophically rising torrent of information, editors and referees should be very careful about this. It is extremely important to add to the still very scanty data on the anatomical and fine structure and morphogenesis of dactylogyrineans. It is best to use for this purpose in vivo observations, photography and drawings (under coverslip) of organs, glands, ducts and musculature, their action at various stages of ontogeny, beginning with larvae. P. J. Gerasev begins now carry out such investigations in our Institute.

A large proportion of the species, sometimes of the genera (for example *Pseudacolpenteron*, *Bychowskyella*), of freshwater dactylogyrineans have been described by their chitinoid structure, with almost complete disregard of other features of their anatomy, or with only fragmentary and sometimes erroneous information about those features. Regardless of the difficulties of studiing some of these details and the impossibility of devoting much time for this purpose in the course of general parasitological work, it is necessary to re-examine from this point of view almost all species of the rich fauna of the USSR (more than 300, or 400 if one adds gyrodactylids). Taking into account the scarcity of specialists, this task can be accomplished only by the joint efforts of general fish parasitologists, and by the involvement of students in this work.

Parasites should not be collected mechanically. One should note the position of worms on the gills and the function and relative position of their attachment organs. It is the inadequacy of such observations, and our imperfect understanding of what a species is, that result in new facts placing under suspicion all earlier information on one of the most dangerous agents of dactylogyrosis, *Dactylogyrus vastator*. Only recently was it possible to determine that this parasite and *D. crassus*, a species of previously uncertain validity, are indeed two separate taxa. Because the majority of investigators did not distinguish between them, it is not clear now which of these two species they dealt with in numerous observations and experiments aimed at the elucidation of the biology and pathogenesis of *D. vastator*. It was uncertain, consequently, against which of the two the therapy and prophylaxis were being developed. Perhaps both species have similar biology, but there might be differences. Taking into account the possibility of such differences, the fact that *D. crassus* appears to be more dangerous than *D. vastator* an that it has been reported from carp (Kollman 1968), one must admit the necessity of repetition of all investigations and experiments for these two species separately.

Many examples of insufficiently studied morphological criteria can be quoted. I will point out only a few. The diagnoses of the genus *Dactylogyrus* and of the subfamily Dactylogyrinae (now a family) state that four eyes are present and that the branches of the intestine are posteriorly confluent. The author found that the branches of the intestine end blindly in *D. robustus* and *D. fallax* and that no eyes are present in a large group of Indian *Dactylogyrus*. There are no other reasons to exclude them from the genus. Many investigators include among generic characters also such features as fusion (or lack of fusion) between the tube of the copulatory organ and its accessory piece, the number of cephalic organs, the presence or absence of a vagina, or the number of marginal hooks (in dactylogyrineans). This is a very formalistic approach to taxonomic criteria. Fusion or otherwise between parts of the copulatory organ, or even the absence of its accessory piece, occurs in closely related species in various genera. The number of glands of the cephalic organ is difficult to establish with certainty in glycerine-gelatine preparations, and many workers indicate them purely "symbolically". A vagina appears to be always present. Not so, however, is its chitinoid lining the absence of which might render it invisible in the preparations. the number of marginal hooks in all dactylogyrineans is 14. The fact that some differential generic diagnoses quote two, or three, or four pairs, results from poor observation and imperfect knowledge of the group. The addition of structures of uncertain nature to marginal hooks or to anchors as their alleged "rudiments" stems from theoretical reasoning and has not enough basis in fact. To find such a basis we need comparative data on action, chemical composition, fine structure and embryogenesis of these "rudiments" and of the marginal hooks and anchors. So far this information is not available. Differences in our understanding of "rudiments", anchors and hooks cause serious divergence in views on classification and phylogeny of the entire class.

The dimensions of various structures are important in the diagnoses of many species. All workers should, therefore, adopt a uniform way of measuring them. By and large, they do so. Hooks and anchors of the haptor, their parts, clamps and their sclerites are measured along a straight line (Gläser 1965, Gussev 1962, 1967, Ergens and Lom 1970 etc.). For the copulatory organ, in addition to total length and diameter of tube in various places, is given also length of tube along its curvature (when it is not straight but forms loops or spirals). The same is true for vaginal armature. However, there are also unwarranted departures from these procedures, e. g., measurements of hooks along their curvatures. This inconsistency makes it impossible to compare many species with structures of similar shape. On the other hand, the length of the anchors of, for example, falcatoid and wunderoid types must not be measured in the same way. Identifications must take into account the wide variability of shape and size of organs in parasites from fishes of different ages (Gussev and Kulemina 1971 a, b).

The type of the life cycle of the parasite and the identity of its host (its group) also in some measure belong to the diagnostic characteristics of narrowly specific monogeneans. This conclusion is based on abundant evidence. It is also reflected in Bykhovsky's (1957) general views on life cycles, occurrence and specificity. It must not be treated as an absolute, but discovery (other than that of a stray specimen) on monogeneans on an unusual host should be brought up for discussion. Unfortunately, many workers pay no attention to such instances and because of this omission are led into various taxonomical and ecological errors (Gussev 1976).

In the "Key" (Gussev 1962), the host lists proved inaccurate for certain species, because of some earlier misidentifications or displacements, such as those of, for example, *Dactylogyrus nanus*, *D. suecicus*, *D. rutili*, *D. nanoides*, *D. difformis*, *D. cornu*, *D. distinguendus*, *D. prostae*, etc. The first to third of these species occur only on *Rutilus rutilus*, the fourth and eight on *Leuciscus cephalus*, the fifth on *Scardinius erythrophthalmus*, the sixth on *Blicca bjoerkna* and *Vimba vimba*, the seventh on *Blicca bjoerkna*, *Vimba vimba* and young *Abramis brama*. These species, as well as *D. difformis*, *D. difformoides* and *D. izjumovae* from *S. erythrophthalmus*, *D. cornu* and *D. cornoides* from *Blicca bjoerkna* and *Vimba vimba*, *D. rarissimus*, *D. crucifer*, *D. caballeroi* and *D. erhardovae* from *Rutilus rutilus*, *D. dirigerus*, *D. elegantis* and *D. ergensi* from *Chondrostoma nasus* can be distinguished only from strongly pressed specimens. Those not acquainted with them or inexperienced investigators can do it only after repeated drawings which allow them to see the differences between closely related species within each of the above groups.

Parasite faunistic studies in the classical style of "dynamics of parasite fauna of fishes" are no longer sufficient. Even though in many regions they are a stage still to be completed, they should now by conducted rather at the population level. This is also required from the practical point of view, in connection with the sharply increased importance of fisheries and fish culture in continental waters and with the necessity of developing mariculture. We can use monogeneans as a very suitable model through which to learn about the processes of the dynamics of parasite populations and about their control, to study this very important ecological problem of our time, particularly under the conditions of the catastrophically rising influence of man on nature. Monogeneans occur on all species and individuals of cyprinid fishes, usually in large numbers, often in groups of several species and genera. We must intensify our work on seasonal and age variability of these parasites, and particularly on their biology, using experimental designs which would expose the influence on them of external enviromental factors; we must develop and apply methods which will produce statistically significant data suitable for mathematical analysis.

It is extremely important that we revive neglected studies on the nature of specificity, on its biochemical basis. Production in the mucus of certain fish species of chemical substances (telergons and parasitepagons) attracting larvae of only "right" species of monogeneans, has a direct bearing on the practical control of monogenoidoses. The

development of such methods and their application is an urgent task for fish parasitologists.

Our studies and interpretation of monogenean material encounters serious difficulties because of lack of uniformity in terminology and nomenclature. There exists great confusion in names of chitinoid structures and their parts. They bear different names not only in different languages, which is understandable, but also in English, in which the majority of relevant papers are published. This applies also to different publications by the same author, or to usage in different groups. Names consisting of two or three words are used for some small structural details, despite the fact that other, concise terms for them exist.

Thus, the heel of a marginal hook is used also for the opposite part of the hook in gyrodactylids. The ligament of the marginal hook is given the name lamella, tendon, domus, posteriorly projecting structure, accessory sclerotized process, filamentous hooklet (FH) loop. It has become difficult to follow the meaning of these terms. If such creativity continues, we will soon cease to understand one another. To restrict it (unfortunately, the Code of Zoological Nomenclature makes no provision for it), the use of Latin names might be, perhaps, the most correct step, assuming its general acceptance. In the absence of such acceptance, however, confusion would only be compounded. It is to avoid it that some single-word English names have already been proposed and occasionally used (Gussev 1976).

This is the proper place to quote what has been stated in the first edition of the code of Zoological Nomenclature, and what fully applies to the difficult problems of methodology, taxonomic criteria and terminology: "Some of our nomenclatural usage has been the result of ignorance, vanity, obstinate insistence on following individual predilections, much like that of languages in general, of national customs, prides and prejudices. Ordinary languages grow spontaneously... but biological nomenclature has to be an exact tool that will convey a precise meaning to all persons in all generations".

The problem of terminology should be included in the programs of international biological organizations with a view to developing general recommendations aimed at limiting this superfluous "inventiveness".

At present at least five systems of numbering are used for marginal hooks. Sometimes hooks are numbered differently in different publications of the same author (sometimes even to different places in the same paper; see Bykhovsky and Gussev 1950, Bykhovsky 1957) or in different monogenean groups (Fig. 1). All these systems are arbitrary until information becomes available on the sequence of appearance of these hooks in the course of embryogenesis. Numeration based on their distribution in larvae, and for this reason most natural, was already proposed 50 years ago (Kulwiec 1927). It would be worth returning to that system, which would remove artificially created difficulties in homologizing the hooks.*

Discrepancies exist among investigators also in terminology referring to more general problems. For example, the concept of parasite specificity is understood in various ways, different from that accepted by the school of ecological parasitology (Dogiel 1962, Bykhovsky 1957). A proposal was made recently to replace it with the term hostality (Ryzhikov 1973). This proposal is difficult to accept. The concept of specificity is solidly entrenched in our and foreign literature. All it needs is to be cleansed from various encrustations and to be kept distinct from the concept of localization, or adaptation to external environmental factors.

There are also differences in interpreting concepts of parasitology and pathology, fish parasitology and fish pathology. There is a tendency to see the first and the third as smaller components of the second and fourth. This is incomprehensible and essentially incorrect. Pathology is a study of processes associated with diseases, while parasitology deals with phenomena associated with the life of one organism in or on, and at the expense of, another. In an historically well "settled" and balanced host-

* During ICOPA IV (Warszaw, 1978) it was decided to count hooks after *Llewellyn* (1963).

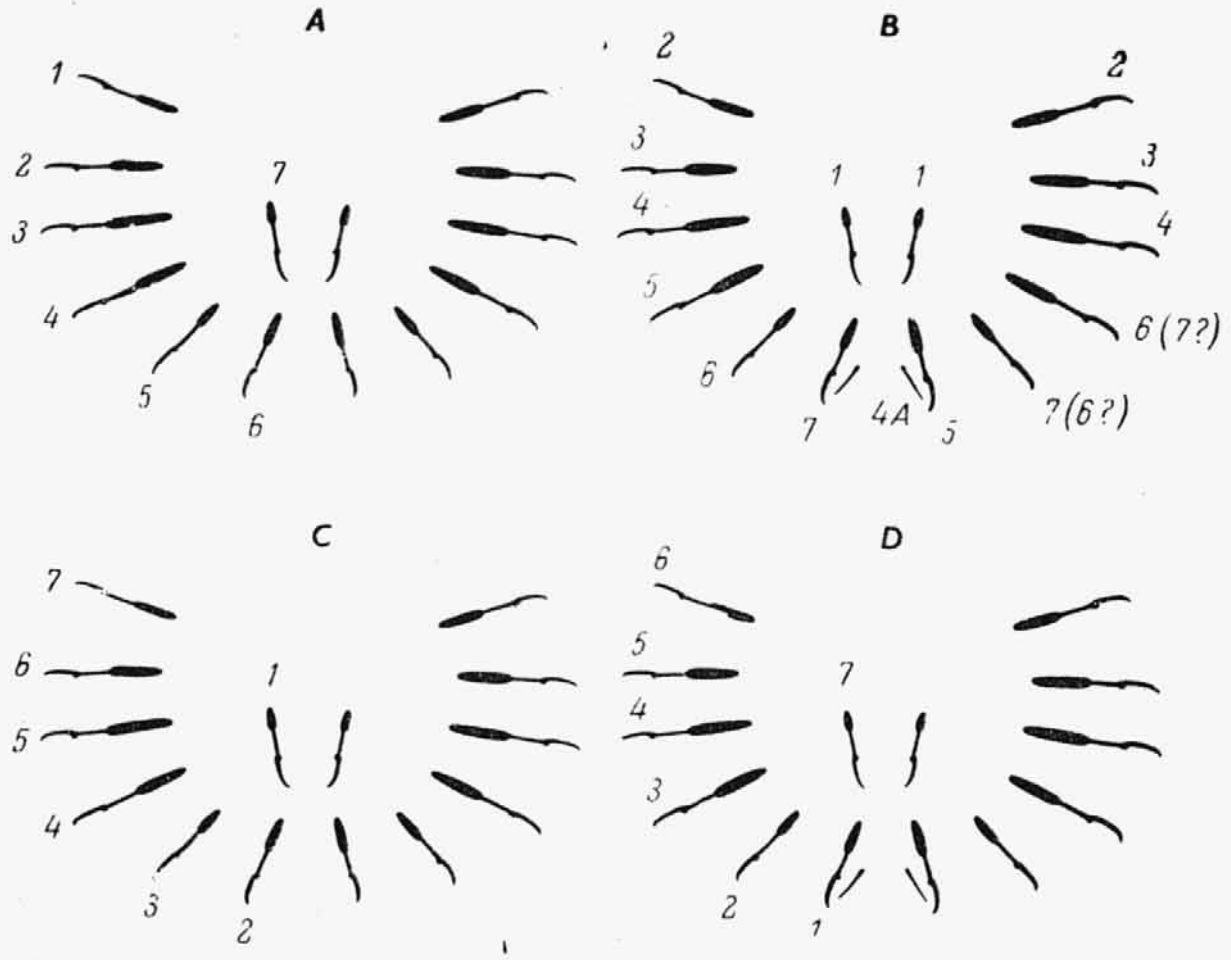

Fig. 1. Numbering of hooks in larvae: A — after Kulwiec 1927 for dactylogyrids and after Llewellyn 1957 for diclidophorideans; B — after Mueller 1936 (left side) and after Mizelle 1936 (right side) for adult Dactylogyridae; C — after Llevellyn 1963 (for different groups) and after Euzet and Ktari 1970 for calceostomatids; D — after Euzet and Ktari 1970 for the others groups (according to personal communication of Dr. A. Lambert) and after Lambert 1975 for Dactylogyrinea.

parasite system usually the parasite causes no harm to the host, which has developed defensive mechanisms. Disease results only when equilibrium is disturbed (as in severe infections, debility of the host, etc.). Many bacteria and viruses are also parasites, yet they are not all (probably very few) pathogenic, and then, most likely, only within a certain range of conditions differing from optimal. It is not by accident that mass diseases of fishes are almost unknown in pure, natural waters, while being common in fish farms.

Most zoologists and parasitologists now share Bykhovsky's view that Monogenoidea and Trematoda are separate classes. Others, however, even the founder of this concept himself, continue to use the traditional term "monogenetic trematodes" in titles and texts of their publications. To avoid such inconsistency we must use the name "monogeneans" or Monogenoidea. (This view was accepted also during ICOPA IV).

It was possible to deal above with only some problems of methodology and work methods, terminology and nomenclature. However, they suffice to show how much has been insufficiently worked out and remains uncoordinated in the study of monogeneans. Being of major theoretical and practical interest, this group calls for more intensive and many-faceted studies. This, in turn, requires augmentation of the ranks of specialists, the accumulation of new, methodologically correct collections of material, their study, and the synthesis of findings and conclusions.

МЕТОДЫ И ТЕРМИНОЛОГИЯ В ИЗУЧЕНИИ МОНОГЕНЕЙ

А. В. Гусев

Резюме. Подчеркивается необходимость точного определения видов при любых работах с организмами, в частности с моногенеями и при фаунистических исследованиях. Это невозможно без соблюдения жестких требований: хорошего качества препаратов, фотографически точных крупно масштабных рисунков и измерений по единой схеме. Даются некоторые практические рекомендации по методике и по учету ряда диагностических критериев, которым противопоставлена формальная оценка признаков отдельными специалистами. Приводятся примеры терминологической путаницы.

REFERENCES

BYKHOVSKAYA I. E., Parazitologicheskoe issledovanie ryb. (Parasitological studies of fishes.) Nauka, Leningrad, 109 pp., 1969. (In Russian.)

BYKHOVSKY B. E., Monogeneticheskie sosalshchiki, ikh sistema i filogeniya. (Monogenetic flukes, their systematics and phylogeny.) Izd. AN SSSR, Leningrad, 509 pp., 1957. (In Russian.)

—, GUSEV A. V., Fam. Diclybothriidae (Monogenoidea) and its systematic position. Parazitol. Sborn. 12: 269—299, 1950. (In Russian.)

DOGIEL V. A., Obshchaya parazitologiya. (General Parasitology.) Izd. Leningr. Univ., 463 pp., 1962. (In Russian.)

ERGENS R., LOM J., Původci parasitárních nemocí ryb (Pathogenes of parasitic diseases of fish.) Academia, Praha, 383 pp., 1970. (In Czech.)

EUZET L., KTARI M. H., Le développement larvaire et la position systématique des Calceostomatides (Monogenea). C. R. Acad. Sc. Paris 271, sér. D: 2332—2335, 1970.

GLÄSER H. J., Zur Kenntnis der Gattung *Dactylogyrus* Diesing, 1850 (Monogenoidea). Z. Parasitenk. 25: 459—484, 1965.

GUSSEV A. V., Monogeneticheskie sosalshchiki (Monogenetic flukes). In: Opredelitel parazitov presnovodnykh ryb SSSR (Key to parasites of fresh-water fishes of the USSR). Moskva—Leningrad: 200—379, 1962. (In Russian.)

—, On morphological criteria and characters in the present systematics of fresh-water Monogenoidea. Parazitologiya 1: 55—66, 1967. (In Russian.)

—, Freshwater Indian Monogenoidea. Principles of systematics, analysis of the world fauna and their evolution. Ind. J. Helm. 25—26: 1 to 241, 1976.

—, Ammonium picrate as a fixative and mounting medium for slides of fish parasites. Zool. Zh. 47: 935—936, 1968. (In Russian.)

—, KULEMINA I. V., Taxonomic characters of some Monogenoidea from hosts of various age. Parazitologiya 5: 162—171, 1971 a. (In Russian.)

—, —, Analysis of variability of characters, behaviour and development's cycle of monogeneans from the age of hosts. Parazitologiya 5: 320—329, 1971b. (In Russian.)

KHOTENOVSKY Y. A., A method for making preparations of diplozoons. Zool. Zh. 53: 1079—1080, 1974. (In Russian.)

KOLLMAN A., *Dactylogyrus crassus* Kulwiec, 1927 (Trematoda, Monogenoidea) auf den Kiemen von Karpfen. Zool. Anz. 180: 43 bis 49, 1968.

KULWIEC Z., Untersuchungen an Arten des Genus *Dactylogyrus* Diesing. Bull. Acad. Polon. Sc. et Lettres Cl. Sc. Math. et Nat., ser. B: 113—144, 1927.

LAMBERT A., Développement post-larvaire d'*Actinocleidus* sp. Mueller, 1937 (Monogenea, Monopisthocotylea, Ancyrocephalidae) Hypothèse de la migration des onchoblastes chez des Dactylogyroidea. C. R. Acad. Sci. Paris, 281, ser. D: 1329—1332, 1975.

LLEWELLYN J., The larvae of some monogenetic trematode parasites of Plymouth fishes. J. Mar. Biol. Ass. U. K. 36: 243—259, 1957.

—, Larvae and larval development of Monogeneans. Adv. Parasitol. 1: 287—326, 1963.

MIZELLE J. D., New species of trematodes from the gills of Illinois fishes. Am. Midl. Nat. 17: 785—806, 1936.

MUELLER J. F., Studies on North American Gyrodactyloidea. Trans. Am. Micr. Soc. 1: 55—72, 1936.

RYZHIKOV K. M., To the question of terminology characterizing helminth-host relationship and ways of analysis of these relationships. In: Probl. obshchey i prikl. gelmintologii. Moskva: 119—123, 1973. (In Russian.)

Received 7 September 1977.

A. V. G., Zoological Institute of the Academy of Sciences of the U.S.S.R.,
199164 Leningrad, U.S.S.R.