ATTI ACCADEMIA NAZIONALE DEI LINCEI

CLASSE SCIENZE FISICHE MATEMATICHE NATURALI

RENDICONTI

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The planarians of the Dugesia lugubris-polychroa group: taxonomic inferences based on cytogenetic and morphologic data

Atti della Accademia Nazionale dei Lincei. Classe di Scienze Fisiche, Matematiche e Naturali. Rendiconti, Serie 8, Vol. **48** (1970), n.3, p. 369–376.

Accademia Nazionale dei Lincei

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Zoologia. — *The planarians of the* Dugesia lugubris–polychroa group: *taxonomic inferences based on cytogenetic and morphologic data* (*). Nota di MARIO BENAZZI, ILEANA PUCCINELLI e RINALDO DEL PAPA, presentata (**) dal Corrisp. M. BENAZZI.

RIASSUNTO. — Gli AA., sulla base di dati citogenetici e della morfologia dell'apparato copulatore, mettono in evidenza l'esistenza, nell'ambito del « gruppo *Dugesia lugubris – polychroa* », di tre specie gemelle, corrispondenti, rispettivamente, ai biotipi A–B–C–D, E–F, G. Ritengono che ai biotipi A–B–C–D si debba attribuire il nome specifico *polychroa* ed a quelli E–F il nome *lugubris*, mentre non credono opportuno, per il momento, assegnare un terzo nome specifico al biotipo G.

Queste specie gemelle (che potrebbero anche chiamarsi semispecie *sensu* Mayr) costituiscono una superspecie, cui gli AA. propongono di assegnare il nome *lugubris*, come quello più comunemente usato per tali planarie.

INTRODUCTION.

With this paper we want to give a new contribution to the problem, which has been discussed for many years, of the specific rank of *Dugesia lugubris* (O. Schmidt) and *D. polychroa* (O. Schmidt), fresh-water planarians which are widely distributed in Europe.

The history of this problem may be summarized as follows. Within the *Planaria torva* Müller, Schmidt (1860) distinguished two new species: *Planaria lugubris* and *P. polychroa* which, after the subdivision of the old *Planaria* genus (Kenk 1930), were attributed to the *Euplanaria*, and then to the *Dugesia* genus. Schmidt based the distinction of the two species on external characters (i.e. the colour, the head shape, the position of the eyes) which lacked any taxonomic value, and on the copulatory system; this was studied, however, on squash preparations, which were not suitable for an exact reconstruction of the organs. For example, in *polychroa* Schmidt admitted the presence of an adenodactyl which following AA. (Chichkoff 1892, etc.) showed to be non existent.

After Schmidt, the most significant contribution was given by Böhmig (1909) who found differential characteristics of the copulatory organs in the two species. Later on we shall discuss these characteristics and also the question raised by Komárek (1926) about a supposed interchange of the two species made by Böhmig. For the moment we must remember that the distinction between *lugubris* and *polychroa* was accepted by some AA. such as Arndt (1926) and Kenk (1930) but denied by others; e.g. Steinmann and Bresslau (1913) for whom the differences between the presumed two species were only

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(*) Lavoro eseguito nell'Istituto di Zoologia dell'Università di Pisa.

(**) Nella seduta del 14 marzo 1970.

quantitative and Wilhelmi (1922) who thought that the description of one species was valid also for the other. Also Funaioli (1951), by means of researches carried out in our laboratory, concluded that the two Schmidt species cannot be accepted since the variability of the morphological characters found in different populations is also present in individuals of the same population. This conclusion, as will be seen, now appears completely justified, because Funaioli examined only specimens of A–B–C–D biotypes which were then the only ones available. In fact, further research carried out by Benazzi (1951–1960) revealed, within the "*D. lugubris polychroa* group", a markedly karyological differentiation (7 biotypes) together with, in some cases, reproductive isolation, which of course suggested again the taxonomic problem. As the main question, Benazzi intended to establish whether the two Schmidt species were karyologically distinguishable and he was able to do this thanks to the kindness of Prof. Reisinger who sent him living specimens collected in the original localities (around Graz, Austria), i.e. the topotypes of the two species. The cytological examination showed that the *polychroa* topotype belongs to biotype A, and the *lugubris* one to biotype F.

CYTOGENETIC DATA.

At this point it is necessary to summarize the karyological research accomplished by Benazzi, which allowed him to distinguish the 7 biotypes, indicated with the first seven letters of the alphabet (for more details see Benazzi 1960, 1963).

Biotype A is diploid ($2n = 8$, $n = 4$) and amphimictic. Biotype B is triploid in the somatic line (12 chromosomes) and hexaploid in the female germ line (12 bivalents in the oocytes). Biotype C is triploid in the somatic line and also in the female line, since the oocytes possess 12 univalents, i.e. they are asynaptic. Biotype D is similar to C but tetraploid instead of triploid. Biotype B–C–D are pseudogamic (i.e. the egg develops ginogenetically) and their male germ line is diploid and meiotic. These first 4 biotypes form a homogeneous series with polyploid evolution starting from biotype A, as is shown by the fact that in all of them the basic set of 4 chromosomes is the same: i.e. a large submetacentric (M) and 3 acrocentrics of different length (A_l, A_m, A_p). These 4 biotypes are, furthermore, interbreeding, although the cross between diploid individuals acting as female and triplo-hexaploid individuals often fail to produce offspring.

Biotypes E, F and G are, on the contrary, chromosomically differentiated and reproductively isolated (figs. 1 and 2).

Biotype E is diploid ($2n = 8$, $n = 4$) but the oocyte and spermatocyte bivalents do not correspond, in length and chiasmata frequency, to those of biotype A. This is confirmed in the corresponding karyotype, which shows that the haploid set is formed by 3 large acrocentric chromosomes of different length, and by a very small one (Benazzi and Puccinelli 1961).

Biotype F has $2n = 6$, $n = 3$ and with all probability originated from biotype E through a Robertsonian translocation, i.e. a centric fusion between two large acrocentrics chromosomes with the formation of a metacentric. In fact, the haploid set of biotype F is formed by a large metacentric, a medium length acrocentric and a very small chromosome.

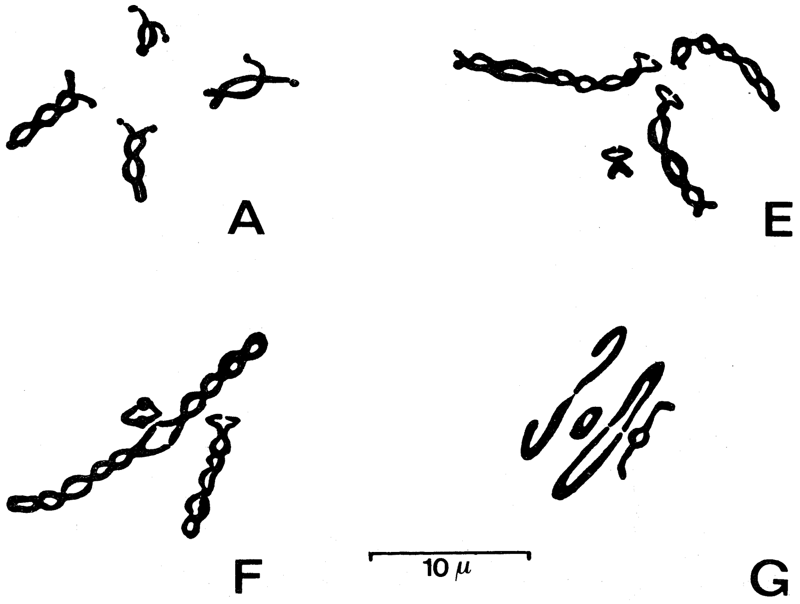

Fig. 1. – Bivalents of the oocytes in biotypes A, E, F, G.

Biotype G is also diploid ($2n = 8$, $n = 4$) but with different bivalent morphology; the haploid set is formed by a large submetacentric chromosome, a chromosome which is a little shorter and more heterobrachial, a medium length subtelocentric and a short acrocentric. This chromosome set is more similar to that of biotype A, from which it may be derived through some chromosomal mutations, e.g. pericentric inversions in chromosomes Al and Am (Benazzi and Puccinelli, *loc. cit.*).

On the basis of these results Benazzi (1963) admitted the existence of 4 sibling species, corresponding, respectively, to biotypes A–B–C–D, E, F, G: " Therefore I think the old distinction between *P. lugubris* and *P. polychroa* in Schmidt's sense should not be retained, because four sibling or incipient species can be recognized. They are reproductively isolated, but not easily distinguishable on morphological grounds. Examination of the copulatory organs of the different biotypes is now in progress in our laboratory " (pag. 410).

However, the karyological similarity between E and F biotypes and the probable origin of the latter from the former by means of a chromosomal mutation (1), suggests a close genetic relationship and the possibility of considering them as a single species. This seems to be confirmed also by new cross-breeding research by Benazzi. In fact, although these two biotypes do not hybrizide (either in nature or in laboratory), a single pair cross E × F gave many offspring from both partners: this shows that sexual isolation may break down in some cases and that between the two biotypes other reproductive barriers do not exist.

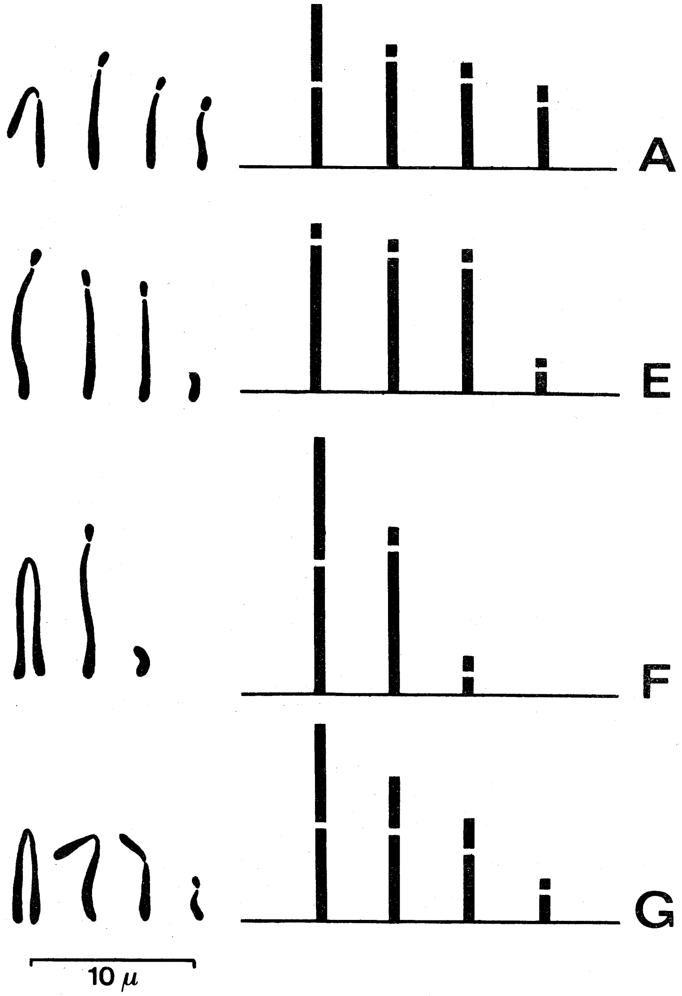

Fig. 2. – Haploid set and idiogram of biotypes A, E, F, G.

On the basis of the above, it seems opportune to admit, within the "*D. lugubris-polychroa* group", not four but three sibling species, well defined

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(1) Besides the centric fusion mentioned above, a slight difference would seem to exist in the centromere position of the smallest chromosome.

from a cytogenetic point of view and represented by biotypes A–B–C–D, E–F, G respectively.

The problem of the possible existence of morphological differences, able to distinguish these sibling species on the basis of the typological species concept, is proposed again on more precise grounds. As we had at hand the topotypes of the two Schmidt species, we concentrated our studies on them. Later we extended our research to specimens coming from different European localities and belonging to different biotypes; this morphological research has been carried out principally by Del Papa.

MORPHOLOGICAL DATA.

First of all, we must say that the external characters (head shape, position of eyes, auricular sense organs) of the topotypes have not furnished elements suitable to distinguish the two species; the *lugubris* topotype is darker, but the differences in colour are without taxonomic value (2).

On the contrary, the morphology of the copulatory system offers, as Böhmig had already seen, significant data (fig. 3).

In the *polychroa* topotype (biotype A) the penis bulb is not clearly separated from the seminal vescicle and the papilla is rather short; also the passage from bulb to papilla is not marked. A narrow horizontal ejeculatory duct starts from the seminal vescicle and after turning at right angles becomes wider at the level of the bulb muscular mass, then narrows again in the papilla. The inner wall of the seminal vescicle is often folded so that the lumen appears irregular.

In the *lugubris* topotype (biotype F) the penis bulb, oval in shape, is large and the papilla is longer. There is a marked separation between the seminal vescicle and the bulb. The ejaculatory duct remains narrow for a longer tract, becoming wider only in a limited area of the bulb, and runs in an antero-posterior direction. The seminal vescicle cavity is more uniform.

We may add that the seminal vescicle in the *polychroa* topotype, at least in most cases, is more dorsal, which determines an initially dorso-ventral direction of the ejaculatory duct and a lateral insertion with respect to its widening. Instead, in the *lugubris* topotype the seminal vescicle is more anterior and therefore the direction of ejaculatory duct is antero-posterior. The openings of the deferents in the seminal vescicle are more anterior in the *polychroa* topotype. The eosinophyl glands inside the ejaculatory duct are usually more abundant in *lugubris*, while in *polychroa* eosinophyl glands are more frequent near the seminal vescicle.

These morphological characters correspond quite well to those shown by Böhmig, who attributed great significance to the presence or absence of a

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(2) The pigmentation, within the «*D. lugubris–polychroa* group», may vary considerably (from dark brown to almost milk white, from uniform to spotted) also in populations of the same biotype; these differences are genetically controlled (Benazzi 1965).

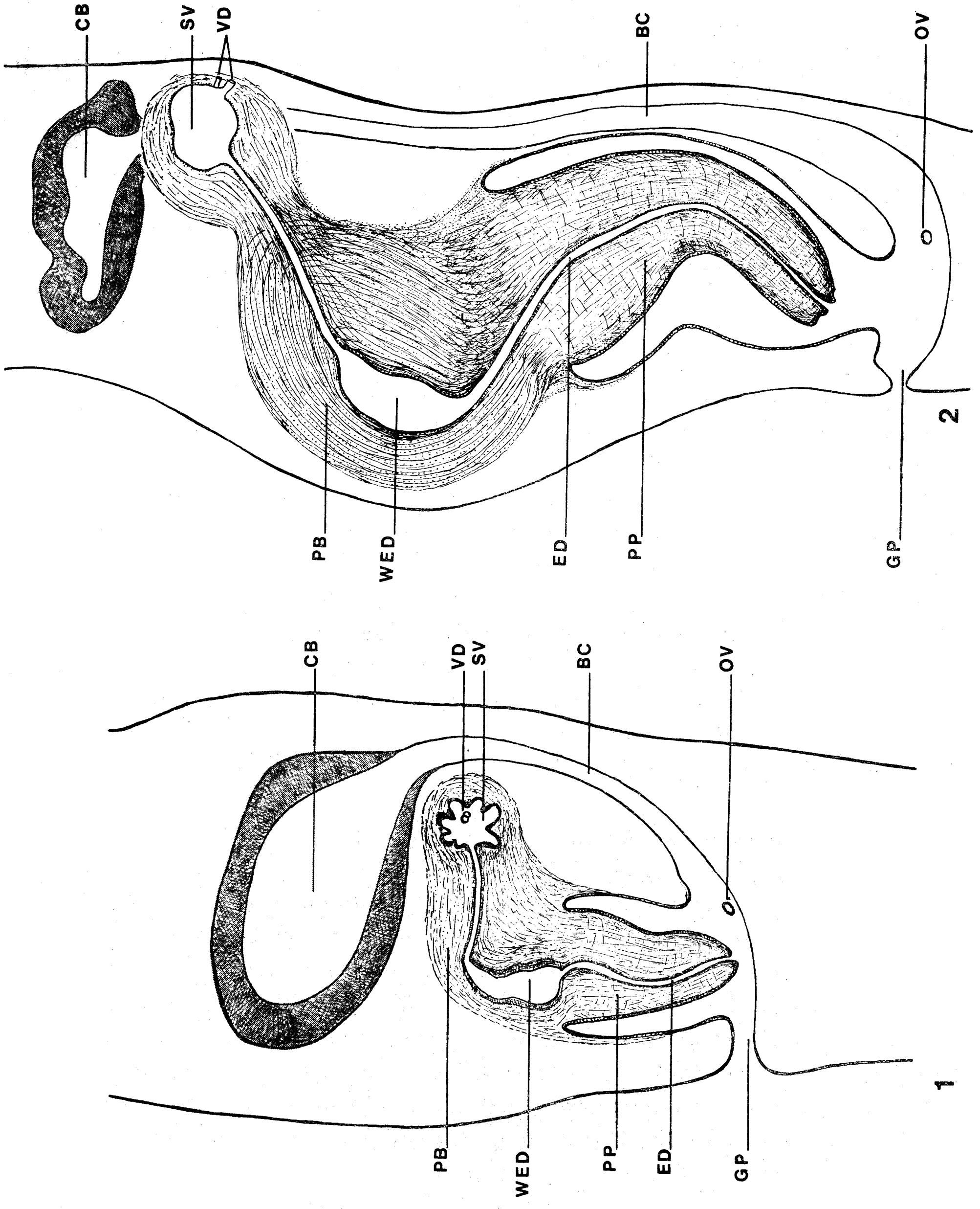

Fig. 3. – Copulatory organs in sagittal section: 1) *polychroa* topotype, 2) *lugubris* topotype.

BC, bursal canal; CB, copulatory bursa; ED, ejaculatory duct; GP, gonopore; OV, oviduct; PB, penis bulb; PP, penis papilla; SV, seminal vesicle; VD, vas deferens; WED, widening of the ejaculatory duct.

marked separation between seminal vescicle and penis bulb: "Mit Rücksicht hierauf habe ich daher entsprechend den Angaben Schmidts diejenigen Individuen, die die scharfe Trennung des Penisbulbus von der Vesicula seminalis aufwiesen, als *Pl. polychroa*, die anderen als *Pl. lugubris* bezeichnet". (Böhmig, 1927, pag. 306).

In our opinion this diversity may be due to the remarkable development of the penis bulb in the *lugubris* topotype, which appears to be one of the most constant and evident characteristics.

Our comparative research on specimens coming from other European localities is still in progress, owing to the variability observed among the different populations. However, even at present we can state the following: the specimens belonging to biotypes A–B–C–D are substantially similar to the *polychroa* topotype and those belonging to biotype E and F are similar to the *lugubris* topotype. Instead biotype G specimens have some of the characters of the *polychroa* topotype and some of the *lugubris* topotype, and furthermore with a certain variability among the different populations examined; therefore, we are not yet able to give a definite judgment about the individuality, from a morphological standpoint, of this biotype.

CONCLUDING REMARKS.

The existence of two sibling species corresponding to biotypes A–B–C–D and E–F respectively, is therefore confirmed in the morphological data. As regards the naming problem it is necessary to keep in mind a fact which has complicated the matter. In fact Komárek (1926) believing he was able to distinguish the two Schmidt species on the basis of external characters stated that Böhmig had interchanged the specimens; therefore the figure of the copulatory system attributed by Böhmig to *polychroa* really corresponded to *lugubris* and vice-versa. Böhmig (1927) replied that he had not made such a mistake, but he admitted that the interchange of the specimens could have been made by Schmidt himself, as in the same locality in which he found *lugubris*, also *polychroa* was found later. So Böhmig recognised that it was necessary to reverse the figures of the copulatory apparatus published by Schmidt and himself: "...dementsprechend sind dann auch di fig. 280 und 284 in Brauers 'Süsswasserfauna' zu vertauchen" (p. 307).

We think that the question is not clear and it is very difficult to ascertain whether the interchange has really been made. The sure data we have are the following:

1) The specimens sent by Prof. Reisinger as *polychroa* topotype belong to biotype A and show a copulatory apparatus without a clear separation between seminal vescicle and penis bulb; according to Böhmig's first description we should consider them as *lugubris* while accepting the interchange they correspond to *polychroa*.

2) The specimens sent as *lugubris* topotype belong to biotype F and show a marked separation between seminal vescicle and penis bulb; therefore,

they would correspond to *polychroa* according to Bohmig's first description, and to *lugubris* if we accept the interchange.

We think it is opportune to accept the second interpretation since Böhmig himself admitted the interchange: therefore, we consider biotypes A–B–C–D as *Dugesia polychroa* and biotypes E–F as *D. lugubris*.

As regards biotype G, we do not think it is necessary at the moment to give it a new specific name. This biotype has only been found in Corsica, Sardinia, Sicily and its karyotype is the most similar to that of biotype A, from which it probably derives. It is worth remembering that in some localities of Corsica and Sardinia we have found also biotype A; this makes our interpretation more likely.

As a final point, we wish to emphasize that the species of the "*D. lugubris-polychroa* group" are primarily defined on cytogenetic data and on reproductive isolation: i.e. on the biological species concept. We must consider them as a group of closely related species (or semispecies *sensu* Mayr) forming a superspecies to which we think it is opportune to give the name *lugubris*, because this name is the most commonly used to indicate these planarians.

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