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G. A. Stocchino^a, G. Corso^a, R. Manconi^a, S. Casu^a & M. Pala^a

^a Dipartimento di Zoologia e Antropologia Biologica dell'Università di Sassari, Sassari, Italy

^b Dipartimento di Zoologia e Antropologia Biologica dell'Università di Sassari, Via Muroni 25, I-07100 Sassari, Italy E-mail:

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Endemic freshwater planarians of Sardinia: Redescription of *Dugesia hepta* (Platyhelminthes, Tricladida) with a comparison of the Mediterranean species of the genus

G. A. STOCCHINO, G. CORSO, R. MANCONI, S. CASU, & M. PALA

Dipartimento di Zoologia e Antropologia Biologica dell'Università di Sassari, Sassari, Italy

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Abstract

The endemic freshwater planarian *Dugesia hepta* from Sardinia was described mainly by karyology and karyotype analysis and by geographic distribution. The present paper reports on the detailed morphological study of the copulatory apparatus and highlights the fact that the exclusive diagnostic characters of *D. hepta* are the shape of the penis papilla, the course and opening of the ejaculatory duct and the openings of the shell glands. A neotype is designated on the basis of a detailed description, and the geographic range of the species is better defined. The two phylogenetically important traits represented by the course and opening of the ejaculatory duct show an unknown condition in the genus *Dugesia*, diverging from the typical one displayed by the other 69 species. A comparative analysis of morphological traits of the 20 Mediterranean species of the genus *Dugesia* was made.

Keywords: *Distribution, Dugesiidae, flatworms, insular fauna, morphology, neotype, taxonomy*

Introduction

Five species of freshwater planarians of the family Dugesiidae are known from Sardinia: *Dugesia benazzii* Lepori, 1951, *D. etrusca monoadenodactyla* Lepori, 1947, *D. sicula* Lepori, 1948, *D. hepta* Pala, Casu and Vacca, 1981, and *D. leporii* Pala, Stocchino, Corso and Casu, 2000 (Lepori 1951; Pala et al. 1980, 1981, 1995, 2000). *D. hepta* and *D. leporii* are endemic to Sardinia, *D. benazzii* and *D. etrusca monoadenodactyla* have a Tyrrhenian distribution, whereas the geographic range of *D. sicula* covers the entire Mediterranean area.

Originally, the diagnostic description of *D. hepta* was given mainly on the basis of karyology and distribution analyses (Pala et al. 1981). The species is characterized by a peculiar chromosomal number, $n=7$; $2n=14$, from which derives the specific epithet, which is unique within the western Palaearctic region. This chromosomal pattern is known only for *D. japonica ryukyuensis* Kawakatsu, Oki, Tamura and Sugino, 1976, and *D. batuensis* Ball, 1970, from eastern Asia (Ball 1970; Kawakatsu et al. 1976).

The original description of *D. hepta* was, however, not exhaustive for the morphological traits at the level of the copulatory apparatus, which are diagnostic at the species level. Furthermore, the species was established without a type series designation, holotype and paratypes were not fixed in the original description, and the type material, not deposited in a collection, was apparently destroyed. It was necessary therefore to designate a neotype among recently collected specimens from the type locality (International Commission on Zoological Nomenclature 1999, Article 75).

A neotype of *D. hepta* is here designated and described together with an update of its geographic range. A detailed morphological analysis of diagnostic characters displayed by the type and comparative material was carried out. A comparison with the other 19 Mediterranean species of the genus was also performed to characterize the relationships of *D. hepta*.

Material and methods

Twenty-two sites were recently surveyed and 22 populations of *Dugesia hepta* were sampled. Specimens were collected in the locality, which is reported in the original description, here considered the type locality, and in all other sites reported in the same paper (Pala et al. 1981). In addition, sampling was extended to other neighbouring hydrographic basins (Figure 1).

Planarians were reared in glass bowls in semi-dark conditions at 18°C and fed with fresh beef liver.

A karyological analysis was carried out on nine specimens by the squashing method described in Vacca et al. (1993). After regenerative processes each specimen was fixed. They were killed in 0.5% acetic acid in order to ensure a relaxed musculature for the morphological study. They were then fixed in Bouin's fluid and embedded in paraffin following the standard methods.

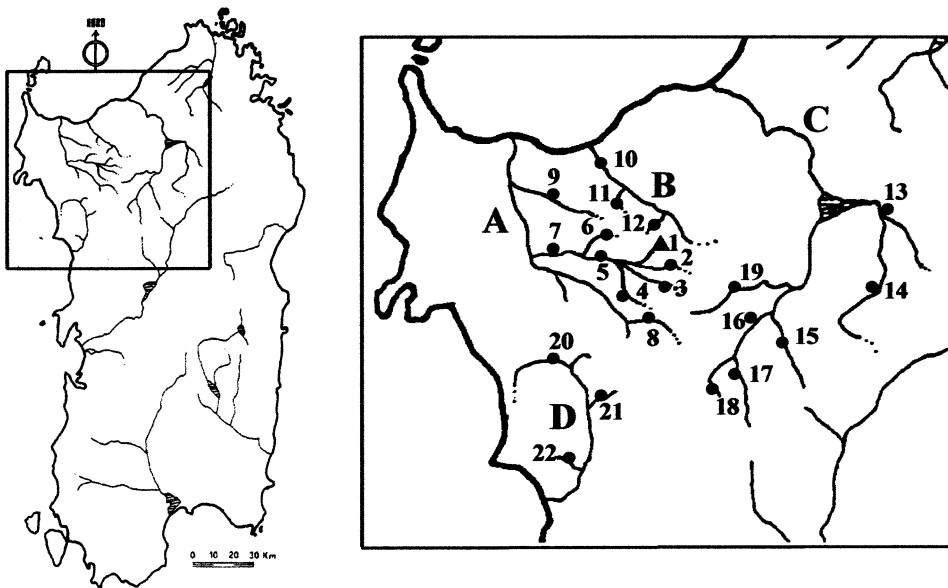


Figure 1. *Dugesia hepta*. Geographic range in four hydrographic basins in NW Sardinia: (A) Rio Mannu di Portotorres; (B) River Silis; (C) River Coghinias; (D) River Temo. Type locality in the Rio Sorgenti San Martino is indicated by (▲). Other localities indicated by (●), and numbers are reported in the text (see Distribution).

Serial sections (5 µm thick) were obtained from the sagittal, frontal and transverse planes. The sections were stained with haematoxylin eosin (Harris) for general morphology. Heidenhain's iron haematoxylin, alcian blue at pH 2.5 (Pearse) or Pasini's reagent were also used to highlight some details of the internal morphology.

The following abbreviations are used in the figures: a, atrium; bc, bursa copulatrix; bg, bulb glands; cb, bursal canal; cg, cement glands; d, diaphragm; ed, ejaculatory duct; fsv, false seminal vesicle; g, gonopore; m, musculature; o, oviduct; pb, penis bulb; pf, penial fold; pfg, penial fold glands; pp, penis papilla; s, spermatophore; sg, shell glands; sv, seminal vesicle; vd, vas deferens.

Systematic account

Suborder TRICLADIDA Lang, 1884

Family DUGESIIDAE Ball, 1974

Genus *Dugesia* Girard, 1850

Dugesia hepta Pala, Casu and Vacca, 1981

(Figures 1–7)

Type material and all specimens, both living and preserved, are at present deposited in the collection of Prof. Maria Pala at the Dipartimento di Zoologia e Antropologia Biologica of the University of Sassari (DIZAB).

Type material

Neotype: DIZAB Pla 3.1, one set of sagittal sections on 88 slides, Rio Sorgenti San Martino (40°41'N, 8°40'E), 18 May 2000, G. A. Stocchino, leg.

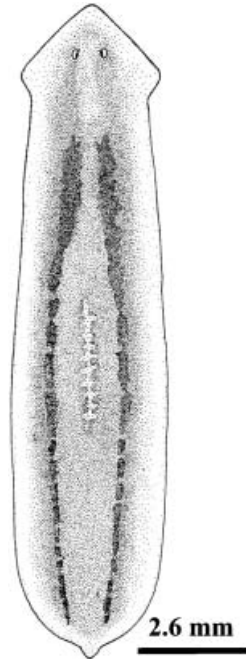


Figure 2. *Dugesia hepta*. External features (from photographs of living specimens).

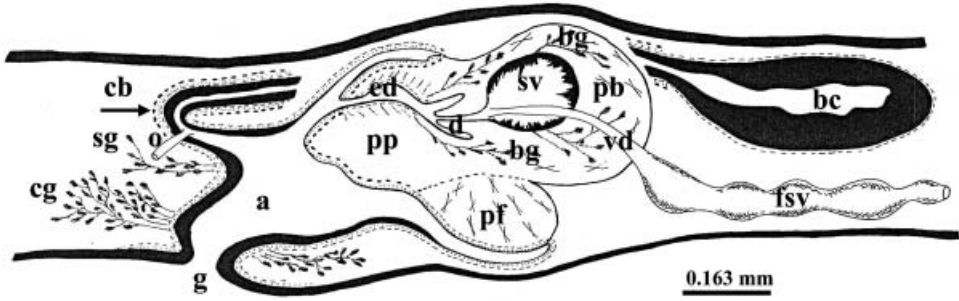


Figure 3. *Dugesia hepta*. Neotype DIZAB Pla 3.1. Sagittal reconstruction of the copulatory apparatus (anterior is to the right).

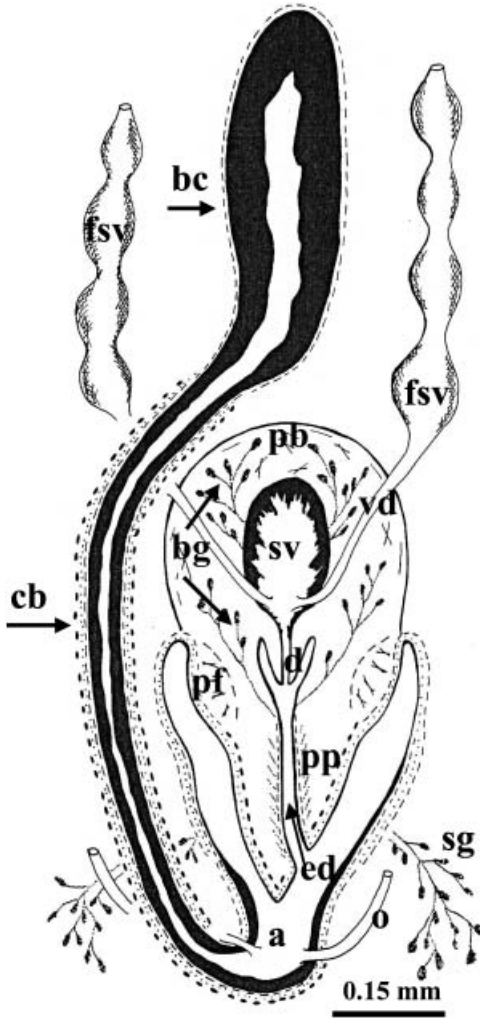


Figure 4. *Dugesia hepta*. DIZAB Pla 3.6. Frontal reconstruction of the copulatory apparatus (anterior is to the top).

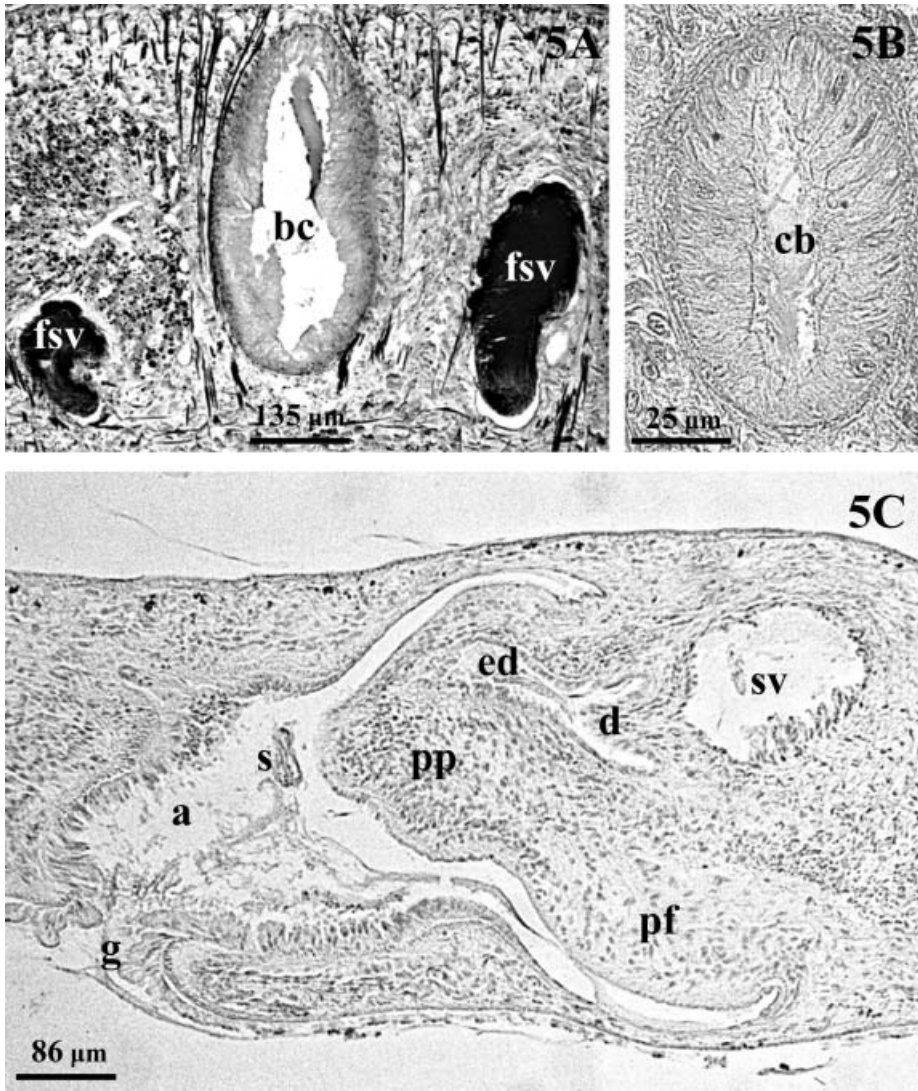


Figure 5. *Dugesia hepta*. Light micrograph sections through the copulatory apparatus. (A) Transverse section of the bursa copulatrix. Note the false seminal vesicles. Heidenhain's iron haematoxylin. (B) Transverse section of the bursal canal. Pasini. (C) Sagittal section showing the penis bulb containing the seminal vesicle, the penis papilla with the ejaculatory duct, the penial fold localized between the penis bulb and the penis papilla. Note a spermatophore in the atrium. Haematoxylin eosin.

Other material examined

DIZAB Pla 3.2, one set of sagittal sections on 68 slides; DIZAB Pla 3.3–5, three sets of transverse sections on 108 slides, 135 slides, 44 slides; DIZAB Pla 3.6, one set of frontal sections on 24 slides (same location as for neotype), 18 May 2000, G. A. Stocchino, leg.

DIZAB Pla 3.7, one set of sagittal sections on 99 slides; DIZAB Pla 3.8, one set of transverse sections on 119 slides; DIZAB Pla 3.9, one set of frontal sections on 47 slides, Rio dei Molini (40°41'N, 8°38'E), 20 June 2001, G. A. Stocchino, leg.

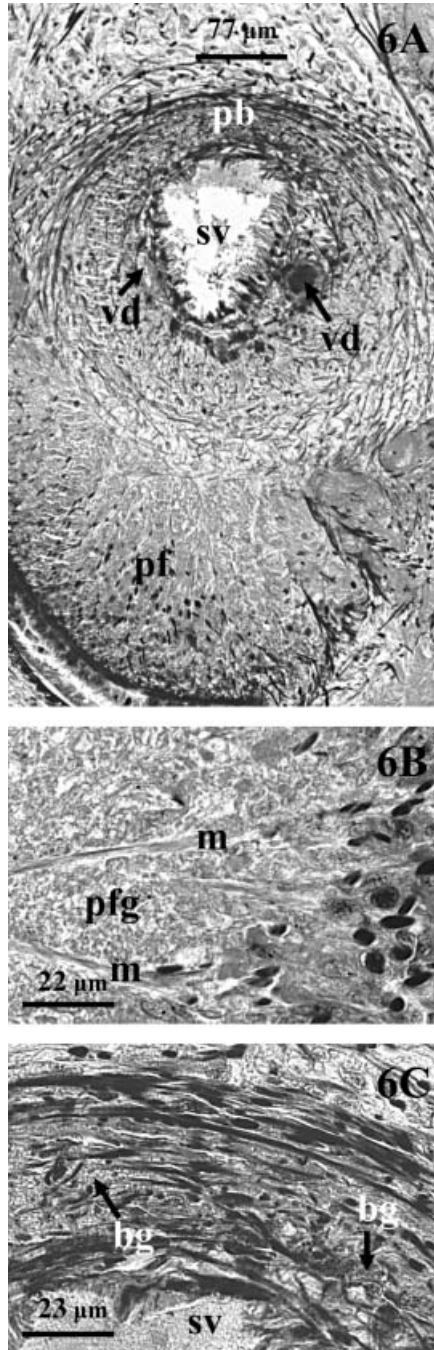


Figure 6. *Dugesia hepta*: light micrograph sections through the copulatory apparatus. (A) transverse section of the penial fold and the penis bulb. Note both vasa deferentia opening in the seminal vesicle. (B) Detail of the penial fold of glandular type. Transverse section. (C) Detail of the penis bulb showing the bulb glands. Transverse section. (A–C) Heidenhain's iron haematoxylin.

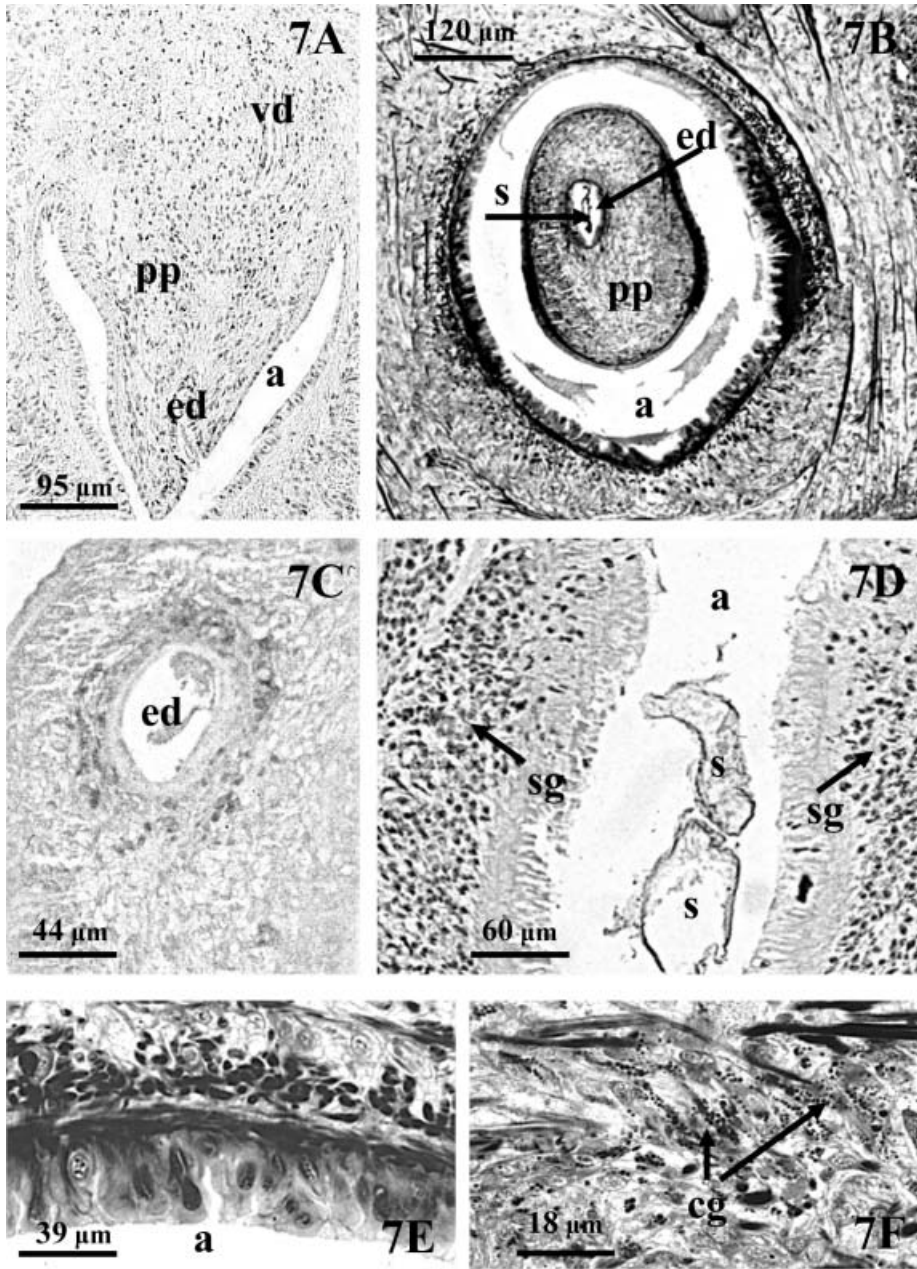


Figure 7. *Dugesia hepta*: light micrograph sections through the copulatory apparatus. (A) Frontal section showing the ejaculatory duct opening laterally on the right with respect to the apex of the penis papilla. Haematoxylin eosin. (B) Transverse section of the penis papilla protruding in the atrium. Note the dorsal position of the ejaculatory duct and the spermatophore in the lumen. Heidenhain's iron haematoxylin. (C) Transverse section of the ejaculatory duct surrounded by numerous glands. Alcian blue. (D) Transverse section of the atrium with spermatophores in the lumen. Note shell glands opening in its centre-lateral area. Haematoxylin eosin. (E) Detail of the atrium showing luminal epithelium. Transverse section. (F) Transverse section of numerous cement glands. (E, F) Heidenhain's iron haematoxylin.

Comparative material

Slides of *Dugesia biblica*, *D. etrusca*, *D. leporii*, and *D. sicula* were examined. For the other species, the comparative analysis refers to data from the original descriptions (see Table I; Benazzi 1946; Lepori 1947, 1948a, 1948b, 1951; Benazzi and Banchetti 1972; Pala et al. 1981, 2000; De Vries and Benazzi 1983; De Vries 1984, 1986, 1988).

Diagnosis

Dugesia hepta is characterized by the peculiar dorsal course of the ejaculatory duct and its opening located laterally on the right with respect to the penis papilla apex; asymmetrical penis papilla with a ventro-lateral penial fold; openings of shell glands localized below the vaginal area of the bursal canal. The species is distinguishable from other dugesiid species by peculiar macroscopic body traits such as an abruptly pointed tail, the less obvious evident retro-cephalic narrowing (“neck”) and the presence, on the surface, of two dorsally situated longitudinal dark brown stripes extending over the whole body length.

Distribution

Dugesia hepta is known from 22 sites in the north-western part of Sardinia. The range of the species is restricted to four hydrographic basins (Figure 1A–D): (A) Rio Mannu di Portotorres basin: 1, Rio Sorgenti San Martino; 2, Rio Montes; 3, Rio s’Iscia; 4, Rio dei Molini; 5, Rio Murrone; 6, Rio Bunnari; 7, Rio Mascari; 8, Rio Badu ‘e se; 9, Rio Logulentu; (B) River Silis basin: 10, River Silis; 11, Rio San Lorenzo; 12, Rio Furrinchesu; (C) River Coghinas basin: 13, Rio Mannu di Pattada; 14, Rio di Oschiri; 15, Rio Buttule; 16, Rio Mannu di Ozieri; 17, Rio Ilde; 18, Rio Santa Lucia; 19, Rio Badde Dianesu; (D) River Temo basin: 20, River Temo; 21, Rio Mulinu; 22, Rio Maggiolsi.

Habitat

Dugesia hepta lives in shallow, clear, running waters on substrates ranging from pebbles, cobbles and boulders to timber and aquatic vegetation. The water level shows notable fluctuations around the year with a temperature of 15–20°C and pH 7–8. Almost all populations were found in small tributaries, except for site 10 in the main course of the River Silis (Figure 1). Large numbers of cocoons were found exclusively during spring. *D. hepta* was found in macrozoobenthic communities composed mainly by other planarians and amphipods, but also by gastropods, benthic insects, and leeches. When associated with other planarians, *D. hepta* was coexistent with *D. sicula* in the River Silis, *D. benazzii* (diploidic and polyploidic biotype) in the Rio Mannu di Portotorres, River Coghinas, River Temo and River Silis, and *Schmidtea polychroa* (biotype A) in the River Silis and Rio Mannu di Portotorres (Vacca et al. 1988, 1993).

Description

All specimens studied showed the peculiar chromosomal number, $n=7$; $2n=14$.

External features. Measured specimens are about 14–17 mm long and 3–4 mm wide. Two eyes are present in the middle of the head. Auricular grooves lack pigment. Five sensory fossae are present on each side of the anterior margin of the head.

Table I. Diagnostic traits of *Dugesia hepta* compared to Mediterranean species of the genus *Dugesia*.

Characters	<i>D. hepta</i>	<i>D. aenigma</i>	<i>D. arcadia</i>	<i>D. ariadnae</i>	<i>D. benaszii</i>	<i>D. biblica</i>	<i>D. brigantii</i>	<i>D. cretica</i>	<i>D. damoe</i>	<i>D. elegans</i>	<i>D. etrusca</i>	<i>D. gonocephala</i> s.s.	<i>D. ilecana</i>	<i>D. leporii</i>	<i>D. ligurientis</i>	<i>D. malickyi</i>	<i>D. minotauros</i>	<i>D. sagitta</i>	<i>D. sicula</i>	<i>D. subtenaculata</i>
Outer pharyngeal musculature																				
Bilayered	+	+	-	-	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	-
Three-layered	-	-	+	+	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	+
Bursal canal																				
Ectal reinforcement	-	+	+	+	?	+	+	+	+	+	-	+	?	+	+	+	+	+	-	+
Opening of the oviducts																				
Symmetrical	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+
Asymmetrical	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-
Penis bulb																				
Well developed	+	-	+	-	+	-	+	+	-	+	+	+	+	+	+	-	+	-	-	-
Weakly developed	-	+	-	+	-	+	-	-	+	-	-	-	-	-	-	+	-	+	+	+
Opening of the vasa deferentia into the seminal vesicle																				
Anterior	-	-	-	-	-	+	-	-	-	+	+	-	-	-	-	-	+	-	+	-
Posterior	+	+	+	+	+	-	+	+	+	-	-	+	+	+	+	+	-	+	-	+
Diaphragm																				
Pointed	+	-	-	-	+	-	-	+	-	-	-	+	-	+	+	-	+	-	-	-
Large	-	+	+	+	-	-	+	-	+	+	+	-	+	-	-	+	-	+	-	+
Small	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-
Shape of the penis papilla																				
Conical	+	-	-	+	-	-	+	+	+	-	+	+	+	-	+	+	+	+	-	-
Blunt	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+
Finger-shaped	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ovoidal	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cylindrical	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
Course of the ejaculatory duct																				
Central	-	+	+	+	+	-	+	+	+	+	+	+	+	-	+	-	+	+	-	+
Dorsal	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ventral	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	+	-	-	+	-

Table I. (Continued).

Characters	<i>D. hepta</i>	<i>D. aenigma</i>	<i>D. arcadia</i>	<i>D. ariadnae</i>	<i>D. benazzii</i>	<i>D. biblica</i>	<i>D. brigantii</i>	<i>D. cretica</i>	<i>D. damoe</i>	<i>D. elegans</i>	<i>D. etrusca</i>	<i>D. gonocephala</i> s.s.	<i>D. ilvana</i>	<i>D. leporii</i>	<i>D. liguriensis</i>	<i>D. malickyi</i>	<i>D. minotauros</i>	<i>D. sagitta</i>	<i>D. sicula</i>	<i>D. subentaculata</i>
Opening of the ejaculatory duct																				
Terminal	-	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+	-	+
Subterminal	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	+	-
Lateral	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Adenodactyls	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
Penial folds																				
Parenchymatic-muscular	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	+	-	-
Parenchymatic-glandular-muscular	+	+	+	+	+	-	+	-	+	+	+	-	+	-	-	-	+	-	-	-
Penial valve	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
Atrium																				
Divided	-	+	-	+	-	+	-	+	+	-	-	+	+	-	-	+	-	-	+	-
Undivided	+	-	+	-	+	-	+	-	-	+	+	-	-	+	+	-	+	+	-	+
Opening of the shell glands																				
Into the bursal canal	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Into the atrium	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chromosomal basic number	$n = 7$	$n = ?$	$n = ?$	$n = ?$	$n = 8$	$n = 8$	$n = 9$	$n = 8$	$n = 8$	$n = ?$	$n = 8$	$n = 8$	$n = 8$	$n = 8$	$n = ?$	$n = ?$	$n = ?$	$n = 8$	$n = 9$	$n = 8$

Width is almost constant along the entire body from the retro-cephalic area to the tail, which makes the retro-cephalic narrowing (“neck”) less obvious than in other *Dugesia* species. The caudal area is abruptly pointed. Two longitudinal dark brown large stripes extending throughout the body length are clearly visible on the dorsal surface, one at each side of the pharynx with proximal portion behind the ovaries (Figure 2).

Colour is light brown dorsally with the exception of a colourless peripheral band (0.5 mm in width), the ventral surface is paler and bears oral and genital pore openings.

Internal features. Inner and outer pharyngeal musculature is bilayered. The ventrally located, paired ovaries in the retro-cephalic region are in contact with the enlarged part of the oviduct (seminal receptacle) in their dorso-lateral area. Oviducts curve toward the ventral area running caudally up to the vaginal area of the bursal canal where they open separately and symmetrically.

The copulatory apparatus with the bursa copulatrix and its canal, the penis, the atrium, the vasa deferentia, and the oviducts, were drawn at the same scale and represented as sagittal and frontal reconstruction (Figures 3, 4). The bursa copulatrix, sac-like in shape, laterally compressed and elongated in the antero-posterior direction, occupies more or less the dorsal half of the body. It is surrounded by a thin longitudinal muscle layer and lined with high secretory cells with basal nuclei (Figure 5A). The bursal canal runs to the left of the copulatory apparatus and opens dorsally in the atrium. Its wall consists of an infranucleate secretory epithelium with cylindrical, ciliated cells. The lumen epithelium is surrounded by a thin inner layer of longitudinal muscles and a thick outer circular one (Figure 5B).

Ripe testes, numerous and well developed, begin at the level of the ovaries. Vitellaria are located in between the testes. The penis, $\pm 630 \mu\text{m}$ long, is formed by the penis bulb and the penis papilla. The large and highly muscular penis bulb, rich in secreting glands, contains a large eccentric seminal vesicle (Figure 6A, C). This vesicle is separated from the ejaculatory duct by a pointed diaphragm and receives the symmetrical openings of the vasa deferentia in its terminal tract (Figures 5C, 6A). At the level of the bursa copulatrix, the vasa deferentia form numerous false seminal vesicles (Figures 3, 4, 5A).

The penis papilla is cone-shaped, has the same length as the bulb and protrudes in the atrium (Figure 5C). The papilla is lined by an infranucleate epithelium below which extends the circular muscular layer.

A penial fold is localized ventro-laterally in the area of transition between the penis bulb and penis papilla. Histologically it consists of parenchymatic glandular tissue surrounded by a well-developed muscular sheet, which in some places penetrates in the parenchyma (Figures 5C, 6A, B).

The ejaculatory duct is situated dorsally and opens laterally on the right side with respect to the penis papilla apex (Figures 5C, 7A, B). This condition makes the penis papilla asymmetrical, with the ventral part greater than the dorsal one. Several glands open along the entire course of the ejaculatory duct (Figure 7C). In all studied specimens collected during spring the ejaculatory duct is filled with spermatophores, sometimes protruding in the atrium (Figures 5D, 7B, D).

The atrium is undivided and opens ventrally by a gonopore (Figure 5C). The atrial cavity, lined by a simple folded epithelium of variable height (Figures 5C, 7B, D, E), receives the secretion of shell and cement glands. The former are few and open just ventrally of the vaginal area of the bursal canal (Figure 7D), whereas the latter are more abundant and open near the gonopore (Figures 3, 7F).

Discussion

Although *Dugesia hepta* is characterized by peculiar characters, it was considered in the original description morphologically very similar to *D. benazzii* (Pala et al. 1981). The present detailed morphological study of the copulatory apparatus highlights the divergence of four main traits between *D. hepta* and *D. benazzii*. The penis papilla is cone-shaped in *D. hepta* and finger-like in *D. benazzii*. The course of the ejaculatory duct is dorsal with lateral opening in *D. hepta*, whereas it is central with apical opening in *D. benazzii*. The openings of the shell glands are localized in the atrium in *D. hepta* and at the level of the bursal canal in *D. benazzii*.

The divergence between *D. hepta* and *D. benazzii* is also supported by a recent molecular analysis (Baguña et al. 1999).

Species belonging to the genus *Dugesia* show two states for the two traits “course of the ejaculatory duct” and “opening of the ejaculatory duct”, namely the course of the ejaculatory duct is central or ventral, and the opening of the ejaculatory duct is terminal or subterminal (Sluys et al. 1998). Our data highlight that a third state for both characters exists in *D. hepta*. The latter species displays an “eccentric-dorsal course of the ejaculatory duct” and a “lateral localization of opening of the ejaculatory duct”. Also, a new condition for the exact location of the openings of the shell glands is reported here. In *D. hepta* they open directly in the atrium, while they are typically localized at the level of the bursal canal in the other species of the genus.

The comparative analysis of morphological traits among the 20 Mediterranean species of the genus *Dugesia* (Table I) indicates that most species are characterized by: (1) outer bilayered pharyngeal musculature (15 species out of 20); (2) presence of ectal reinforcement (15 species out of 20); (3) symmetrical openings of oviducts (18 species out of 20); (4) posterior openings of vasa deferentia (15 species out of 20); (5) conical shape of the papilla (12 species out of 20); (6) central course of the ejaculatory duct (15 species out of 20); (7) terminal opening of the ejaculatory duct (16 species out of 20); (8) presence of structures strictly related to the penial apparatus (16 species out of 20) such as penial folds, adenodactyls and penial valves.

A penial fold is present in 14 species out of 20. The fold can be single or multiple, but situated in variable positions. Histologically, they belong to two types: a parenchymatic-muscular type in *D. gonocephala* s.s., *D. malickyi* and *D. sagitta* (De Vries 1984, 1986) and a parenchymatic-muscular-glandular type shared by most other species (*D. aenigma*, *D. arcadia*, *D. ariadnae*, *D. benazzii*, *D. brigantii*, *D. damoe*, *D. elegans*, *D. etrusca*, *D. hepta*, *D. ilvana*, and *D. minotauros*) (Benazzi 1946; Lepori 1948a, 1951; De Vries and Benazzi 1983; De Vries 1984, 1988).

The adenodactyl only occurs in *D. cretica* (De Vries 1984, 1988) and indicates the folds, with a variable position with respect to the penial papilla, characterized by the constant presence of a lumen.

The penial valve is a peculiar fold, entirely surrounding the basal portion of the penial papilla and it is exclusively known from *D. leporii* (Pala et al. 2000).

Only four species, out of the 20 considered, namely *D. biblica*, *D. liguriensis*, *D. sicula*, and *D. subtentaculata*, bear a penial apparatus lacking annexes (Lepori 1948b; Benazzi and Banchetti 1972; De Vries 1986, 1988).

Among morphological traits, supporting the process of diversification of Mediterranean *Dugesia* species, it seems that the evolutionary novelties, acting as physical constraints during copula, occurred mainly in the penial apparatus at the level of its strictly related structures, matching the lock-and-key hypothesis of Shapiro and Porter (1989) well. The

morphological divergence in this taxon suggests that the process of speciation occurred by reproductive isolation due to the physical incompatibility of partners. The anatomical divergence of the copulatory apparatus traits involved the complex mating behaviour during courtship and copula of these flatworms to allow the reciprocal exchange of sperm (Proctor et al. 1995; Vreys and Michiels 1997, 1998; Vreys et al. 1997).

From a biogeographic point of view, the Mediterranean species of *Dugesia* occur mainly in insular or coastal freshwaters (Lepori 1948a; Pala et al. 1981, 2000; De Vries and Benazzi 1983; De Vries 1984, 1988). The genus *Dugesia* is represented in the circum-Mediterranean area by species with a high level of endemism (70%). Therefore most species show a restricted geographic range, such as a single water course as in the case of *D. leporii* and *D. brigantii*, or water courses of a few hydrographic basins as in the case of *D. hepta*. It seems that their restricted geographic ranges reflect the geological history of hydrographic basin in coastal and insular areas and their long-term geographic isolation. The typical, low dispersal potential of planarians together with the condition of spatial discontinuity of freshwater courses matches a condition of geographic isolation. A process of allopatric speciation of these planarians is strongly supported by their fragmented habitat, the present high endemism values and the morphological divergence in copulatory traits. *D. hepta* represents a good model of this condition: it is endemic to an island, with a range restricted to a few water courses in NW Sardinia, the main traits of its copulatory apparatus are peculiar and different from the other Sardinian and circum-Mediterranean species, and finally its karyological status is unique in the entire western Palaearctic region.

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References

- Baguña J, Carranza S, Pala M, Ribera C, Giribet G, Arredo MA, Ribas M, Riutort M. 1999. From morphology and karyology to molecules. New methods for taxonomical identification of asexual populations of freshwater planarians. A tribute to Professor Mario Benazzi. *Italian Journal of Zoology* 66:207–214.
- Ball IR. 1970. Freshwater triclad (Turbellaria, Tricladida) from the Oriental region. *Zoological Journal of the Linnean Society* 49:271–294.
- Benazzi M. 1946. Sopra una nuova planaria d'acqua dolce. *Archivio Zoologico Italiano* 31:93–102.
- Benazzi M, Banchetti R. 1972. Descrizione di *Dugesia biblica*, nuova microspecie del gruppo *Dugesia gonocephala*, trovata nel fiume Giordano (Israele). *Atti della Società Toscana di Scienze Naturali Memorie Serie B* 79:83–91.
- De Vries EJ. 1984. On the species of the *Dugesia gonocephala* group (Platyhelminthes, Turbellaria, Tricladida) from Greece. *Bijdragen tot de Dierkunde* 54(1):101–126.
- De Vries EJ. 1986. On the taxonomic status of *Dugesia gonocephala* and *Dugesia subtentaculata* (Turbellaria, Tricladida, Paludicola). *Journal of Zoology (London)* 209:43–59.
- De Vries EJ. 1988. Further contributions to the taxonomy and biogeography of the subgenus *Dugesia* (Platyhelminthes, Tricladida, Paludicola) in the Mediterranean region and the Middle East. *Israel Journal of Zoology* 35:109–136.
- De Vries EJ, Benazzi M. 1983. *Dugesia brigantii* n. sp., a freshwater planarian found in an Italian cave. *Bollettino di Zoologia* 50:263–268.

- International Commission on Zoological Nomenclature 1999. International code of zoological nomenclature. 4th ed. London: International Trust for Zoological Nomenclature. 306 p.
- Kawakatsu M, Oki I, Tamura S, Sugino H. 1976. Studies on the morphology, karyology and taxonomy of the Japanese freshwater planarian *Dugesia japonica* Ichikawa and Kawakatsu, with a description of a new subspecies, *Dugesia japonica ryukyensis* subsp. nov. Bulletin of Fuji Women's College 14:81–126.
- Lepori NG. 1947. Descrizione di *Dugesia etrusca monoadenodactyla* nuova razza di planaria di acqua dolce. Monitore Zoologico Italiano 56:35–44.
- Lepori NG. 1948a. Descrizione di *Dugesia ilvana* Benazzi n. sp. di Planaria d'acqua dolce dell'Isola d'Elba. Archivio Zoologico Italiano 33:183–193.
- Lepori NG. 1948b. Descrizione di *Dugesia sicula*, nuova sp. di Triclade d'acqua dolce dei dintorni di Catania. Archivio Zoologico Italiano 33:461–472.
- Lepori NG. 1951. Sulle caratteristiche morfologiche e sulla posizione sistematica della planaria di Sardegna e Corsica già ascritta a *Dugesia gonocephala* (Dugès). Atti Soc Toscana Sci Nat Mem Ser B 58:1–22. .
- Pala M, Casu S, Vacca RA. 1980. Rinvenimento di una Planaria ascrivibile a *Dugesia etrusca monoadenodactyla* Lepori (Turbellaria, Tricladida) nell'Isola di Molara (Sardegna). Bollettino della Societa Sarda di Scienze Naturali 19:177–181.
- Pala M, Casu S, Vacca RA. 1981. *Dugesia hepta*, nuova specie di planaria d'acqua dolce di Sardegna appartenente alla superspecie *Dugesia gonocephala* (Dugès) (Turbellaria, Tricladida). Bollettino della Societa Sarda di Scienze Naturali 20:97–107.
- Pala M, Stocchino GA, Corso G, Casu S. 2000. A new species of freshwater planarian belonging to the genus *Dugesia* (Platyhelminthes, Tricladida) from Sardinia. Italian Journal of Zoology 67:379–383.
- Pala M, Vacca RA, Casu S, Stocchino GA. 1995. The freshwater planarian *Dugesia sicula* Lepori from Sardinia (Platyhelminthes, Tricladida). Hydrobiologia 310:151–156.
- Proctor HC, Baker RL, Gwynne DT. 1995. Mating behaviour and spermatophore morphology: a comparative test of the female-choice hypothesis. Canadian Journal of Zoology 73:2010–2020.
- Shapiro AM, Porter AH. 1989. The lock-and-key hypothesis: evolutionary and biosystematic interpretation of insect genitalia. Annual Review of Entomology 34:231–245.
- Sluys R, Kawakatsu M, Winsor L. 1998. The genus *Dugesia* in Australia, with its phylogenetic analysis and historical biogeography (Platyhelminthes, Tricladida, Dugesidae). Zoologica Scripta 27:273–289.
- Vacca RA, Casu S, Pala M. 1988. Popolamento planariologico dei fiumi del Nord Sardegna. 1. I cariotipi delle planarie d'acqua dolce del gruppo *Dugesia gonocephala* (Turbellaria, Tricladida) presenti nel fiume Silis (Sassari). Bollettino della Societa Sarda di Scienze Naturali 26:131–147.
- Vacca RA, Casu S, Pala M. 1993. Popolamento planariologico dei fiumi del Nord Sardegna. 2. I cariotipi dei Tricladi d'acqua dolce rinvenuti nel bacino idrografico del fiume Coghinas. Bollettino della Societa Sarda di Scienze Naturali 29:59–73.
- Vreys C, Michiels NK. 1997. Flatworms flatten to size up each other. Proceedings of the Royal Society of London Series B 264:1559–1564.
- Vreys C, Michiels NK. 1998. Sperm trading by volume in a hermaphroditic flatworm with mutual penis intromission. Animal Behaviour 56:777–785.
- Vreys C, Schockaert ER, Michiels NK. 1997. Unusual pre-copulatory behaviour in the hermaphroditic planarian flatworm, *Dugesia gonocephala* (Tricladida, Paludicola). Ethology 103:208–221.