



TANDONIA TOTEVI (WIKTOR, 1975) (PULMONATA: MILACIDAE) IN BULGARIA AND NORTH EASTERN GREECE. REDESCRIPTION

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ABSTRACT: New materials of *Tandonia totevi* (Wiktor, 1975) from Bulgaria have enabled the authors to prepare a detailed redescription and to distinguish the species from those from southern Greece which have been confused with *T. totevi* for almost 30 years. From the southern Greek Islands, the Peloponnese and the island of Euboea at least three more species need to be described and discriminated from *T. totevi*. The spermatophores of *T. totevi* from Bulgaria are described here for the first time, as is an intra-integumental glandular secreting organ of unknown function. The first description of spermatozoa of *T. totevi* is included. The first photographs of living specimens and the first observations on the behaviour and ecology are provided.

KEY WORDS: *Tandonia totevi*, Milacidae, slugs, Bulgaria, Greece, anatomy, redescription, biogeography, ecology

INTRODUCTION

This paper is dedicated to Prof. Dr. ANDRZEJ WIKTOR, to honour the scientist who inspired all of us, our teacher and personal friend, who found *Tandonia totevi* (Wiktor, 1975) 44 years ago and described the species in 1975. For a newly detected intra-integumental organ described in this work, we have coined the term “Wiktor’s pocket organ” for common use, to honour Prof. Dr. ANDRZEJ WIKTOR, who has set the mile stones in modern research of slugs almost worldwide.

The genus *Tandonia* Lessona et Pollonera, 1882 (Stylommatophora: Parmacelloidea: Milacidae) comprises species of very different size: from comparably small (*Tandonia piriniana* Wiktor, 1983, 20 mm total length when preserved) to very large (*T. totevi* (Wiktor, 1975), 136 mm total length when pre-

served). Many species of the genus are known only from their original descriptions. The descriptions are often based on scarce and not well preserved material of immature specimens. For the majority of species information on bionomics is missing. Out of 33 described species, 22 holotypes (WIKTOR 1987a) are not available for research in collections anymore. Despite the comprehensive monographic work (WIKTOR 1987a), the systematic position and taxonomy of many species remain unclear.

We came across *T. totevi* only by accident when researching the genus *Limax* sensu lato in Bulgaria. The first results of our work concerning *T. totevi* are presented here. Besides the first finds of the species in Bulgaria by A. WIKTOR in 1967 (see chapter: History of research) the only known samples in Bulgaria were

collected by I. K. DEDOV in 1997, 22 years after the first description. Until the 2009 record in Bulgaria by D. G. GEORGIEV there obviously was no systematic search for, or research on, this species in Bulgaria at all since A. WIKTOR had first detected the species. In 2009 A. CHAUSHEV collected a very good series of topotypes at the locus typicus at Devin.

Unfortunately *T. totevi* was originally collected as subadult and juvenile specimens only. Most charac-

ters of the species were described from only a single specimen (holotype) in the early (subadult) stage and from seven juvenile paratypes. Therefore it is necessary to redescribe the species from adult specimens from Bulgaria to prevent further confusion with other large species of the genus *Tandonia*, especially from the territory of Greece.

HISTORY OF RESEARCH

On the 21st of May 1967 A. WIKTOR collected an unknown species of the genus *Milax* at Devin, Bulgaria at an anthropogene site (eight specimens only). In 1975 he described the species as *Milax (Subamalia) totevi* in honour of his friend and companion during fieldtrips DIMITR TOTEV PETKOV (WIKTOR 1975). The holotype (no. MP 459) and the paratypes are stored in the collection of the Museum of Natural History, Wroclaw University. Catalogue numbers of paratypes have never been published. In July 1980 W. J. M. MAASSEN (personal communication) collected a single specimen of an unknown milacid near the ancient ruins of Philippi, Greece. WIKTOR determined the specimen as *T. totevi* in 1985 and included the record in his Milacidae monograph (WIKTOR 1987). The specimen is stored in the private collection of W. J. M. MAASSEN, Echt, The Netherlands. Next year WIKTOR (1981) published his new genus-level classification of Milacidae where *Milax (Subamalia) totevi* Wiktor, 1975 was classified as *Tandonia totevi* (WIKTOR 1975). A year later MYLONAS (1982) published his paper on the terrestrial molluscs of the Cyclades. All slug identifications were done by WIKTOR. *T. totevi* is mentioned for the island of Kimolos. It is the first mention of the taxon so far south in Greece, 550 km south of the type locality. In 1983 WIKTOR published his important paper on Bulgarian slugs. No further specimens of the species had been found in Bulgaria until that date. In 1987 WIKTOR (1987a) published his systematic monograph of Milacidae. Interestingly, he mentioned 14 specimens examined for *T. totevi* but did not mention the specimens from Kimolos, Greece, which he had identified five years earlier. He also mentioned "specimens" from Philippi, Greece, collected by W. J. M. MAASSEN. Moreover WIKTOR (1987b) concerned about the significance of spermatophores for the milacid classification. Until that date spermatophores of 16 species out of 47 species of Milacidae were known. WIKTOR et al. (1994) published a comprehensive work on the slugs of the Southern Aegean Islands. The authors pointed out that *T. totevi* was much more variable there than the specimens known at the time from Bulgaria and

northeastern Greece. Discrimination of *T. totevi* from *T. cretica* (Simroth, 1885) without examination of the spermatophore was referred to as almost impossible. In 1997 WIKTOR published his paper on the endemism of slugs of the Balkan Peninsula, and expressed doubts regarding the knowledge of distribution of *T. totevi*. On the 4th August 1997 I. K. DEDOV (personal communication) collected two juvenile specimens of an unknown milacid in the valley of Lukovitsa River between the villages Shiroka Laka and Gela, Bulgaria. Because the male genitalia were not developed he could not assign his specimens to either *T. totevi* or *T. sowerbyi* (Férussac, 1823). The specimens are stored as *Tandonia* sp. in I. DEDOV's collection, Inv.-no. 359/A-B. In 2001, after a long delay in the publishing process (six years!), WIKTOR (2001) published his summarising work on the slugs of Greece, mentioning a total of 98 *T. totevi* of which most were collected from the Island of Crete, some from northeastern Greece, Philippi, the islands of Kimolos, Kythira, Karpathos and Kasos. In September 2009 D. GEORGIEV discovered a new population of *T. totevi* near Plovdiv, Maritza River and our team started an ad hoc research project. In December 2009 A. CHAUSHEV collected a valuable series of topotypes of *T. totevi* at Devin. In the summer 2010, two preserved juveniles of *Tandonia* sp., collected in the valley of Lukovitsa River between the villages Shiroka Laka and Gela in 1997 by I. K. DEDOV, were re-examined and definitely identified by U. E. SCHNEPPAT as *T. totevi* sensu Wiktor, 1975. The specimens were compared with the juvenile samples from the Devin and Plovdiv populations as well as with the voucher specimens of *T. sowerbyi* in different developmental stages from the island of Ischia, Italy. In February 2011 a single specimen of *T. totevi*, collected by W. J. M. MAASSEN from Philippi, northeastern Greece and determined by A. WIKTOR as *T. totevi* in 1985 was re-examined. The original determination by WIKTOR (1987a) proved to be correct. All important characters were identical with those of the specimens from the two populations in Bulgaria.



MATERIAL AND METHODS

Except two specimens collected by I. K. DEDOV in 1997, preserved in 75% ethanol, and one additional specimen (preserved in 70% ethanol) from Philippi, Greece, in the collection of W. J. M. MAASSEN, all the studied specimens from Bulgaria were collected by D. G. GEORGIEV near Plovdiv and his student A. CHAUSHEV at Devin between 2009 and 2010 or are the offspring produced in captivity at BNM. In order to protect the species at the Plovdiv site, we did not collect animals during our (GEORGIEV, SCHNEPPAT & KNECHTLE) three recent visits in April 2010. The majority of specimens were sent alive from Plovdiv to BNM - Chur, Switzerland and were kept there in plastic containers and vivaria for different length of time, depending on their age and condition. Some specimens were kept by D. G. GEORGIEV in Plovdiv for behavioural observations and were subsequently released at the collecting site near Plovdiv after several weeks. The slugs were kept at temperatures varying from 8 to 20°C. We tried to breed the species in captivity, but with little success. Nevertheless, eight juveniles from the first generation offspring are currently in captivity at BNM for further research.

Killing and preserving specimens followed NITZ et al. (2009). Dissection procedure followed WIKTOR (1987, 2000). The slime colour was tested with the "paper towel-method" which was developed by the team of Task - Force - Limax/TFL during the course of investigations since 1985. We used a piece of white kitchen paper tissue, wiping off slime from different parts of the integument, which makes it possible to distinguish colours. In addition we observed colouration of the killing liquid and we checked the colour of the secretions from freshly killed animals in a white porcelain dish.

After we detected the intra-integumental organ described in this study, we chose specimen BNM 59145 from the Plovdiv population for micro-morphological and histological examination. This specimen was preserved in 96% ethanol and then transferred into 75% ethanol. The whole integument was carefully cut out with a pointed scalpel blade. The tissue was dehydrated in a graded ethanol series (75%, 80%, 85%, 90%, 96% and 100%) at room temperature for at least 3 h at each concentration. Following dehydration the tissue sample was transferred into the first and second bath of ROTO-Histol (Roth) at 35°C for 12 h each to remove ethanol and then transferred to other baths of Paraplast X-TRA (Roth) for 12 h each at 60°C prior to embedding. The samples were embedded in an embedding frame with Paraplast X-TRA (Roth) at 60°C. After cooling down to room temperature the block was cut with a rotation microtome (Zeiss Microm HM 330) into a series of 10 µm sections. We cut serial transverse sections through the

dorsal body wall containing the postpallial pocket organ. The sections were stretched in a water bath at 60°C and mounted in oriented series of four sections per slide on microscopic glass slides. After subsequent drying for at least ten minutes on a heating pad, the slides were stored. Staining was performed with Mayer's standard hemalum and eosine. To reconstruct the interior dimensions and form of the organ we took digital microscopic pictures of each section of the postpallial pocket organ and stacked layers of varying transparency using Adobe Photoshop.

Aggregated clumps of a whitish material detected inside the bursa copulatrix of two preserved specimens (BNM 59945 & BNM 59946), were provisionally interpreted as "sperm packages", analogous to those found in Limacidae. These were carefully removed, measured with callipers, and stored in 75% ethanol. In specimen BNM 59525 from the Plovdiv population a huge whitish mass was detected inside the bursa copulatrix. After removing this clump in one piece from the bursa lumen, it was measured with callipers and transferred into a 1% sodium-chloride solution, in which it was dissected with thin pointed forceps and dissection needles. Most of this material was then stored in 75% ethanol. We later placed fragments of the material from all three sources mentioned in phosphate buffer (0.1 M Sørensen K/Na buffer pH 7) and reduced the material to small pieces with dissection needles. We examined the preparations unstained or stained with methylene blue (2% in borate buffer pH 8) or toluidine blue (0.5% in 0.1 M Sørensen K/Na buffer pH 7). When dissecting the fragment of BNM 59525 under a dissection microscope we found a knot of spermatophores. The outer layers of whitish aggregations were cleaned away from the knot with thin pointed forceps, dissection needles and fine hair brushes and then placed in a fresh 1% sodium-chloride solution to soften the spermatophores and to swell the attached aggregations that remained on and between the knotted spermatophores. After we took apart the knot, the separated spermatophores were soaked in 1% sodium-chloride solution for two more days and then cleaned under a dissecting microscope with very fine hair brushes (nos. 0 and 00). Due to the very fragile nature of the five spermatophores found knotted with each other it was impossible to separate them without damage (in two spermatophores small fragments were broken off but were preserved). In one spermatophore the anterior portion and in the other the "head" of the anterior section were missing. In one the posterior end was found to be damaged. Measurements were taken with callipers while the spermatophores were in a relaxed position in 1% sodium-chloride solution. Subsequent preservation of the spermatophores was in a graded

ethanol series (30%, 50%, 60%, 70% and 75%) to avoid collapsing the lumen of the tunicles. In a few cases we could not prevent them from breaking along cracks in the tunicles.

In this project no phylogenetic studies were carried out, but we preserved tissue samples from several specimens from the populations at Devin (locus typicus) and Plovdiv for further research. The state of preservation and age of the voucher specimens from the valley of Lukovitsa River and “Philippi” in Greece unfortunately precluded taking samples for further DNA sequence analysis. The treatment of the samples followed NITZ et al. (2009). The samples are stored in the BNM collection.

All morphological investigations generally followed the instructions in NITZ et al. (2009).

In total we examined 43 specimens of *T. totevi* sensu WIKTOR (1975) from three localities in Bulgaria and from one locality in northeastern Greece.

1. Four adults and one juvenile specimen (kept in captivity and later studied as adult) from the locus typicus at Devin, Nastan city area, northern edge of town, Western Rhodope Mountains, Province of Smoljan, Bulgaria, 21°42'39"N, 24°25'08"E, 786 m a.s.l., (BNM 59943–59947) collected on 27–30th December 2009 by ANTON CHAUSHEV, Devin.
2. Two juvenile specimens from the valley of Lukovitsa River, Turlata Ridge, between the villages Shiroka Laka and Gela, Western Rhodope Mountains, Province of Smoljan, Bulgaria, 41°39'55.46"N 24°34'27.66"E, 1300 m a.s.l. (Coll. I. DEDOV Nr. 359/A–B) collected on 4th August 1997 by IVAILO K. DEDOV, Sofia.
3. 21 adult specimens from the environs of Plovdiv, left bank of Maritza River, Upper Thracian Plain, Province of Plovdiv, Bulgaria, 42°09'16.22"N 24°43'19.00"E, 164 m a.s.l., (BNM 59145, BNM 59525–BNM 59530, BNM 59615–BNM 59626) collected on 15th September 2009, 10th November 2009 and on 4th December 2009 by DILIAN GEORGIEV, Plovdiv. Additionally one of these animals (series BNM 59616–59622) laid a cluster of eight eggs (BNM 60083/A–H; Fig. 9E) detected in a vivarium at BNM on 22nd February 2010 (laid about three to four weeks earlier); eight juveniles of F1 generation hatched from these on the same day (BNM 62199–62206).
4. One specimen from a site previously mentioned by WIKTOR (1987a) as the “ancient ruins of Philippi/Filippi”. This citation turned out to be incorrect

(W. J. M. MAASSEN personal communication). Neither Philippi (the ancient ruins) nor Filippi (a small village 4.5 km NE of the ancient ruins of Philippi) can be regarded as the correct location. Due to the long time elapsed since the collection (31 years!) the collector could not remember the site exactly. When discussing the circumstances W. J. M. MAASSEN remembered that he had not collected in the area for longer than an hour and from this collecting trip he had also brought *Theodoxus* sp. Therefore it is most likely the site along the little stream near “Lydia’s Baptism”, about 1.25 km northwest of the ancient ruins of Philippi. It is the only site with open water in the whole area. It belongs to the Administrative Division of East Macedonia and Thrace, Prefecture of Kavala, southwest of the village Lydia, Greece. The estimated coordinates are 41°01'N and 24°16'E, 54 m a.s.l. The specimen was collected in July 1980. Below this particular site is still referred to as “Philippi”. The specimen mentioned was examined and determined by WIKTOR in 1985 (WIKTOR 1987a, 2001, WIKTOR et al. 1994).

For comparison we had a large series of preserved voucher specimens of *Tandonia* spp. (= *T. totevi* sensu WIKTOR after 1982) and *T. cretica* (Simroth, 1885), collected on the islands of Crete and Rhodes by W. J. M. MAASSEN in 1979–1992. Some of the specimens of this series had been determined by A. WIKTOR. We used very good drawings of male genital anatomy and spermatophores of different *Tandonia* spp. which were collected on the island of Euboea and the Peloponnese and at that time it was uncertain whether these were *T. totevi* or another species. The drawings were done by T. de WINTER, Leiden. Large series of further samples of Milacidae spp. from the Balkans and elsewhere in the Mediterranean are in the collection of U. E. SCHNEPPAT at BNM, Chur. Many of the specimens were collected recently by G. WONDRAK, U. SCHNEPPAT, F. KNECHTLE, I. K. DEDOV and D. GEORGIEV. All specimens catalogued with BNM numbers are deposited in U. SCHNEPPAT’s collection at BNM, Chur, Switzerland. The two specimens mentioned from the valley of Lukovitsa River, Bulgaria, Collection I. K. DEDOV, are kept in his private collection in Sofia, Bulgaria. The single specimen mentioned from “Philippi”, Greece, is part of the private collection of W. J. M. MAASSEN, Echt, The Netherlands and is kept there, as well as other voucher specimens (*Tandonia* spp.) mentioned in this paper from the islands of Crete and Rhodes.

REDESCRIPTION

DIAGNOSIS

A very large slug and the largest species of the genus *Tandonia* and of the family Milacidae. Fully adult

specimens with visible and open gonopore range from 137 to 185 mm total length when alive and from 75 to 136 mm when preserved. The external appearance, at least in some specimens, resembles that of



very large specimens of *Limacus flavus* (Linnaeus, 1758). The keel is poorly developed, at the posterior end of dorsum only and sometimes even totally missing. The horseshoe-shaped groove on the mantle is often barely visible in live and preserved specimens. The pigmentation of back and mantle varies from grey-olive to dark olive-brown in live specimens and can darken markedly after preservation. In many specimens a creamy whitish, sometimes pale yellowish to pale orange keel-stripe is easily visible, extending to the posterior edge of mantle, but it may also be inconspicuous or even missing. The very short keel, when developed at all, is dirty olive-brownish. The spotting of mantle and dorsum is variable. Some individuals are not spotted or spots are hardly visible. In all specimens the posterior portion of penis has the shape of a spool and its largest diameter is 2.0–2.5 times that of epiphallus. The epiphallus in all adult specimens is of almost uniform cylindrical shape, at least 2.6–3.5 times the length of entire penis and always bent anteriorly. The epiphallus is connected with the anterior and posterior portion of penis by strong connective

tissue between the penis and vas deferens, similar to that known in Limacidae. This tissue is criss-crossed by haemolymph vessels. The bursa copulatrix is large and tapers into its duct towards vagina without a distinct narrowing at its posterior end. Accessory glands around the vagina in all adult specimens dissected are not “indistinct grape-like structures or irregular lump” (WIKTOR 1987a). They form a flat, broad, smooth and almost seamless rounded belt of bright orange to brick-red colour around the vagina without any distinct surface structure. The structure as described by WIKTOR (1975) is found in subadult specimens only when the glands are not fully developed.

The spermatophores are of unique size and shape among all known members of the genus and family. They are 40–42 mm long and almost bare of accessory spines and spikes. Their size and form are additional unique features distinguishing *T. totevi* from all other species of the genus which were confused with the species and were collected on southern Greek Islands (WIKTOR 2001) and elsewhere in Greece (T. DE WINTER and W. J. M. MAASSEN pers. com.).

DESCRIPTION

BODY MEASUREMENTS

All the measurements were taken from fully adult specimens. Measurements of preserved specimens are given in parentheses. Measurements of specimens from the Devin population are marked with D, those from the Plovdiv population with P and the single specimen from “Philippi”, Greece with PG:

Weight: D 8.0–11.5 g; P up to 31.5 g (6.0–24.0 g); PG (10.0 g)

Total length: D up to 137.0 mm (84.5–100.5 mm); P up to 185.0 mm (75.0–136.0 mm); PG (78.5 mm)

Sole length: D up to 134.0 mm (81.0–95.0 mm); P up to 183.0 mm (72–128 mm); PG (76.0 mm)

Mantle length: D up to 37.0 mm (22.5–26.5 mm); P up to 52.0 mm (12.9–36.0 mm); PG (28.5 mm)

Dorsum length: D up to 85.0 mm (51.0–66.0 mm); P up to 112.0 mm (42.5–84.0 mm); PG (48.0 mm)

Keel length: D (4.0–16.0 mm); P (3.0–16.5 mm)

Mantle width: D (14.5–16.6 mm); P (12.0–22.0 mm); PG (14.8 mm)

Body width at about mid length: D (14.3–17.1 mm); P (13.1–27.1 mm); PG (15.2 mm)

Sole width: D (11.0–13.8 mm); P (10.0–19.0 mm); PG (12.0 mm)

Body height: D (13.3–15.5 mm); P (12.4–23.0 mm); PG (15.5 mm)

Vertical diameter of gonopore: D (2.5–3.5 mm); P (1.5–5.2 mm), PG (2.5 mm)

Horizontal diameter of gonopore: D (2.0–3.5 mm); P (1.0–3.1 mm); PG (2.2 mm)

Specimens from the Plovdiv population may grow to a markedly larger size than those from the Devin population. So far, no comparative series from the population at Lukovitsa River and “Philippi”, Greece have been measured.

COLORATION

The overall pigmentation of the back and mantle varies from light grey-olive with a yellowish tinge (Plovdiv population) to dark olive-brown with a rusty tinge (Devin population) in live specimens (Figs 1A–E, 2A, B, 3A, B). In preserved material the general pigmentation may darken markedly almost to a dark chocolate brown (Fig. 4C). Most specimens from the Devin population are conspicuously darker than those from other known populations (Fig. 1E). However, in all specimens examined, both live and preserved, black pigment was absent. This does not conform with the original description (WIKTOR 1975).

The mantle in live specimens does not show clear light dotting mentioned by WIKTOR (1975). The light spotting might be a preservation artefact. The spots can be inconspicuous or even missing in live as well in preserved animals and in the field at night, if present at all, may pass unnoticed in the light of a torch.

We found that under magnification many of the light dots turned out to be scars, as was the case in most specimens from the Plovdiv population. The scars showed distinct characteristics of bite marks, like those found in several aggressive species of *Limax*. We never observed intra-specific aggression between specimens

of *T. totevi* but the site near Plovdiv holds also a population of the aggressive *Limax* cf. *graecus* Simroth, 1889 co-occurring with *T. totevi*; the many light bite marks may have been caused by this species. However, we could not observe any interaction between the species in the field. This interpretation is confirmed by the many identical bite marks we found in specimens of *Limax* cf. *graecus* from the same site as well as from sev-

eral other sites in Bulgaria. Furthermore, the eight juveniles (BNM 62199–62206) hatched in captivity and still kept alive at BNM do not bear any visible bite marks.

The area surrounding the pneumostome is always devoid of dark pigmentation. In specimens from the Plovdiv population it ranged from creamy white to very pale yellow (Fig. 3A, B). In specimens from the



Fig. 1. *Tandonia totevi*: A – adult specimen BNM 59530, Plovdiv, Maritza River, in its habitat; B – specimen BNM 59145, Plovdiv, Maritza River, secreting milky-white defensive mucus after irritation; C – specimens from series BNM 59525–59530, Plovdiv, Maritza River, pre-copulation behaviour; D – same specimens as in C copulating at night; E – topotype BNM 59945, Devin Town, post copula with still swollen gonopore and visible “papilla”

Devin population it was pale yellow to pale orange (Fig. 2A, B).

Some of the examined specimens show dozens of small pale bite marks also outside the mantle; these pale dots vary in brightness and arrangement. Most dots have an overlay of the general pigmentation and

are not well visible at first sight. Clearly light dots are often arranged along the keel stripe and towards the sole (Fig. 3A). Most specimens at our disposal show a distinct keel stripe along the mid-dorsum (Fig. 3B), but this can be very inconspicuous and narrow (Fig. 2B) or even missing. This stripe varies from creamy



Fig. 2. *Tandonia totevi*. A–C – three views of senile adult BNM 59944, topotype from Devin Town, Nastan city area

white to pale yellow or even pale orange colour and is at most 1.0–2.5 mm wide at mid-dorsum. It was creamy white in the specimens from the Plovdiv population (Fig. 3B). In the specimens from the Devin population it ranged from pale yellow to pale orange. The small keel, when present at all, is always of a dirty

olive and mottled pigmentation. The sides below the mantle, the nape and the lower parts of the head, mouth and tentacles are always light. One specimen from Devin had its mouth flaps brick red. The upper parts of head and ommatophores are greyish olive of varying intensity. This pigmentation is composed of

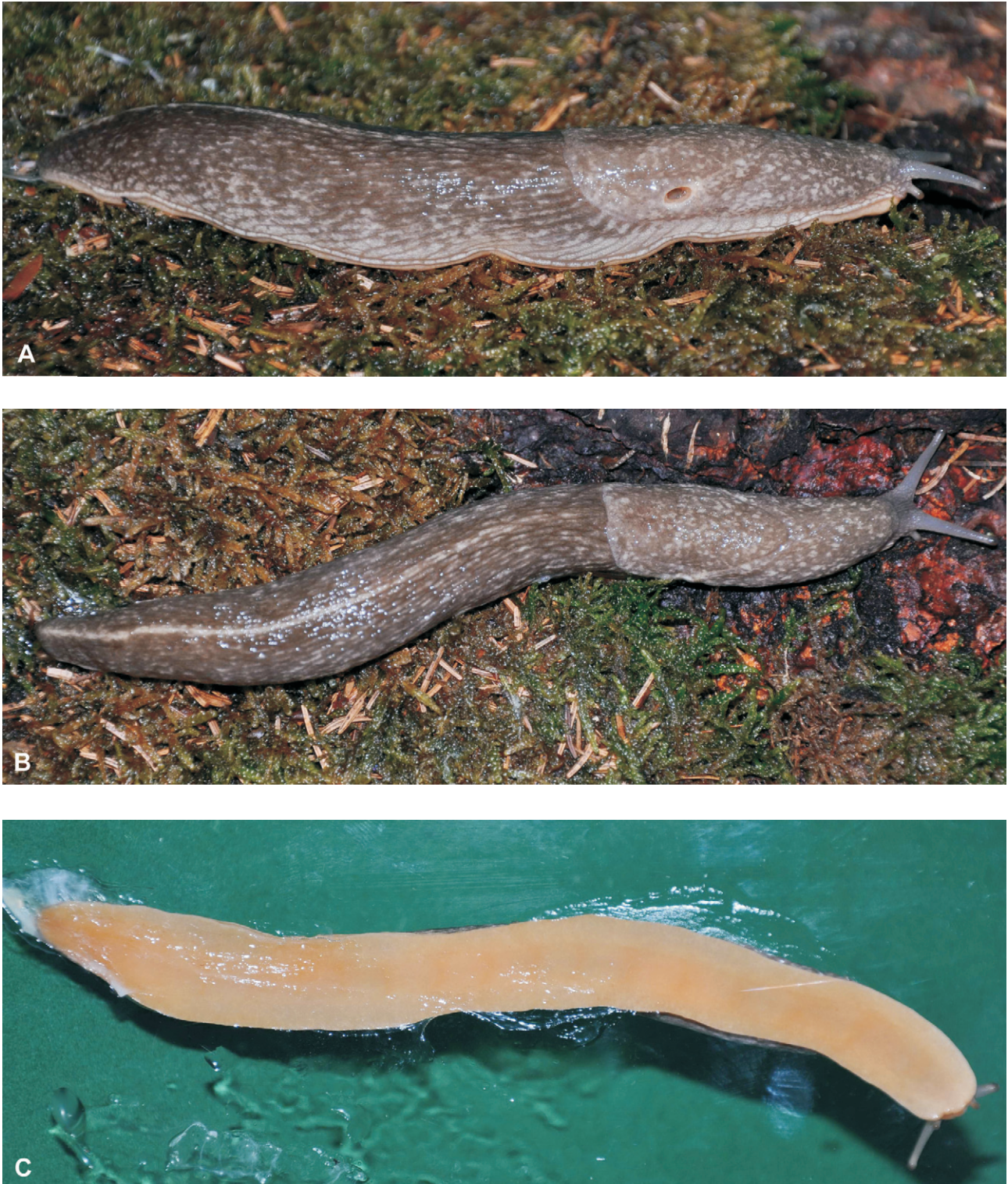


Fig. 3. *Tandonia totevi*: A–C – three views of adult BNM 59145, from Plovdiv, Maritza River (views as in Fig. 2; C – specimen showing normal clear and milky-white defensive mucus at the same time)

fine olive pigment dots which are best visible when the ommatophores are fully everted. The tentacles and mouth have no pigment dots.

Both in live and in preserved specimens the seam of the sole is of cream-yellowish to bright orange colour (Fig. 2A, B) without any dark pigmentation from the anterior almost to the posterior end. Depending on the total length of adult specimens, which varies much, the posterior 10–15 mm of the seam are pigmented first with single dark olive to dark brown spots which soon coalesce to form a uniform dark seam around the end of the body.

The Devin population shows an evenly light orange to orange sole (Fig. 2C), without any visible darker pigment. This character remains in preserved specimens and may change slightly by fading. A single juvenile (BNM 59947) raised from the Devin population had a pale yellow sole until subadult. The Plovdiv population has the sole colour ranging from light yellow (Fig. 3C) to bright yellow, also lacking dark pigment. We never found a specimen with an orange hue in this population.

MUCUS

At first sight in the field the animals are of an almost “dry” appearance (Fig. 1A). Glistening of mucus and slime trails are often inconspicuous, a fact that was also observed in captive animals. Most of our observations were on captive animals; the laboratory conditions may have influenced their behaviour and mucus secretion and quality. Remarkably, *T. totevi* is fast to become habituated to captivity conditions. Already after a few days in captivity, it stops reacting with immediate contractions anymore. Nevertheless we observed properties of the mucus, which with few exceptions only, was always absolutely transparent and colourless, contrary to WIKTOR’s (1975) description. In several cases we observed the animals being covered with a very liquid, sometimes dripping mucus. We interpreted this as a behaviour analogous to “sweating” at higher temperatures. The animals moving on glass produced a transparent and very sticky mucus (Fig. 3C) which, even when dry, was not iridescent. In one case we observed copulation slime trails. This slime was very sticky and iridescent when dry after a few days. When handled shortly after transport (from Bulgaria to BNM, Switzerland) the slugs secreted very thick, sticky and transparent mucus from the dorsum and mantle (see LUCHTEL & DEYRUP-OLSEN 1991, DEYRUP-OLSEN & LUCHTEL 1998). This sort of mucus was not produced anymore after several days in captivity and it seemed that the slugs had become accustomed to handling and captive conditions.

In only one case a specimen from the Plovdiv population secreted well visible milky white defensive mucus (BNM 59145) (Figs 1B, 3C). Preparatory to taking

a photograph, the slug was taken out of its box by hand and, immediately after it was placed on a piece of bark and moss, it secreted this kind of mucus from its dorsum and sides. Seen under magnification the whitish opaque mucus came from the pores in the form of very thin whitish threads and spread patchily beneath the normal mucus layer of the integument. When touched, the mucus was still liquid and seemed to move under the usual thin translucent and colourless mucus layer of the integument, but upon reaching the surface of this layer it immediately became viscous. Later the milky white mucus moved to the posterior end of the body and obviously mixed with the normal slime trail (Fig. 3C). On three occasions we observed a milky defensive mucus in specimens from the Devin population but this was much less intensive in colour. Contrary to the observations on *Deroceras reticulatum* (O. F. Müller, 1774), *T. totevi* never secreted milky white defensive substances when being killed.

MANTLE STRUCTURES

The mantle surface in live individuals is of very fine crenellations, giving an impression of small intra-integumental “bubbles”. This minute surface structure cannot be seen in preserved specimens. The horseshoe-shaped groove observed in live specimens is very shallow and its visibility depends on individual pigmentation and light conditions. Sometimes it is barely visible, as it is not completely developed near the posterior end of the mantle, especially in preserved specimens with overall dark pigmentation and in those which have darkened after killing and preservation. The pneumostome is surrounded by a distinct ring-like structure which is entirely smooth and without any additional structures. The width and visibility of the pneumostome vary in live and in preserved specimens and also individually. They depend on the size of the pneumostome and physiological condition. The “slit” of the pneumostome in all specimens does not end in the lumen of the pneumostome but runs anteriorly to at least the dorsal edge of pneumostome. The position of pneumostome varies little. In preserved adult specimens it is located from 7.5 to 12.0 mm from the posterior margin of mantle (33.3–36.7% of the mantle length) and from 3.2 to 6.0 mm posterior to the middle of the mantle length (12.8–24% of the mantle length). The posterior margin of the mantle is not tightly attached to the integument. The posterior free mantle flap covers the anterior integument-wrinkles (Fig. 4A, B) as well as the slit-like transverse openings of the postpallial pocket organ. In live animals as well as in contracted specimens this posterior mantle flap is smoothly rounded (Fig. 4B). It shows a shallow indentation pointing anteriorly when specimens are fully extended (Fig. 4A). In its centre a narrow flap points posteriorly, which is

only visible in extended specimens. These characters are best visible under magnification and with good lighting. In alcohol-preserved specimens these special structures are often not well visible because of shrinkage, the remains of coagulated slime, or because of disturbance of the structures while handling the dead specimen during preservation and dissection.

INTEGUMENT STRUCTURES

The number of grooves between rows of integument wrinkles, from the midline of back to the slit of pneumostome, varies more widely in our series than formerly reported: Devin, $n = 4$, 16–19 grooves;

Plovdiv, $n = 14$, 15–21 grooves; valley of the Lukovitsa River $n = 2$, 20–21 grooves; “Philippi”, Greece $n = 1$, 17 grooves. The total variation is 15–21 grooves. In our series, the most common number of grooves is 16–21 (21 grooves in the holotype). At least for *T. totevi* this variable character cannot be regarded as a diagnostic feature.

The surface texture and the width of wrinkles in live specimens vary depending on their position. The wrinkle rows from the head to the 7th–9th row posterior to the pneumostome slit are entirely flat. They are smooth and wide (1.5–2.0 mm) at the head end and remain so to the peripodial wrinkle. The wrinkle rows from the 8th to the 10th behind the pneumostome

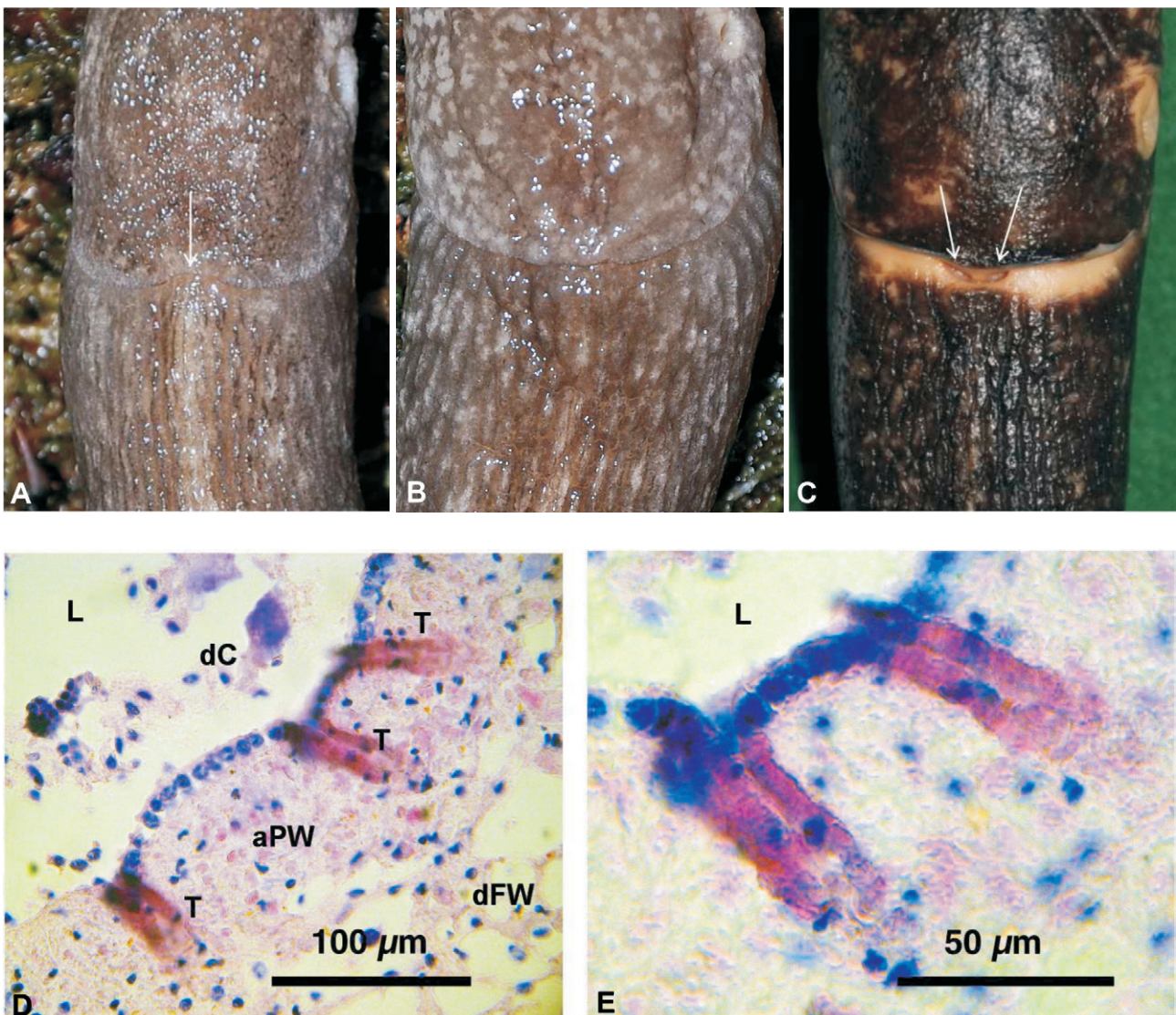


Fig. 4. *Tandonia totevi*: A–C – region of dorsally situated postpallial pocket organ: A – topotype BNM 59947 alive and fully extended; B – specimen BNM 59145 from Plovdiv, Maritza River much contracted (in A and B posterior mantle flap completely covers postpallial pocket organ); C – topotype BNM 59944 preserved, with mantle flap shrunken in ethanol and partly raised anteriorly (both slits of postpallial pocket organ visible and open); D–E – postpallial pocket organ, 10 μm thick frontal section, Meyer’s standard hemalum-eosine stain: D – anterior wall of left pocket with tubular glands; E – same as D at higher magnification. aPW – anterior wall of the pocket; dC – detached cells; dFW – dorsal body wall; L – lumen of the pocket; T – tubules

slit show almost the same surface structure as the mantle and are slightly narrower (1.0–1.5 mm). All these wrinkles are covered by very fine crenellations which, under magnification, seem to move like small bubbles or granules. This might depend on differing pressure of interstitial fluid and/or movement of intra-integumentally flowing haemolymph in the integumental lacuna system. In preserved specimens this structure vanishes almost completely but can be partly preserved in the posterior portion of the dorsum. Single wrinkles are comparatively long (14–20 mm) in the anterior part of the dorsum posterior to the mantle. The general structure of the ridges of the wrinkles on the dorsum has been described as “small” (WIKTOR 1975), or “delicate, very narrow, elongate” (WIKTOR 1987a). These characteristics were found in preserved specimens only. In this state at least some wrinkles appeared to be ridged, which normally is an artefact of preservation and shrinkage but was also observed in few specimens shortly before their natural death, when the pressure of interstitial fluid had collapsed. In fully mature live, healthy specimens all rows of wrinkles are well separated by a distinct and clearly visible groove line. The wrinkles are slightly arched and never ridged.

The keel has been described as short, only 10 mm in length (WIKTOR 1975, 1987a). In ten adult specimens we found the keel discernible from the neighbouring dorsal wrinkle rows, with a length of 3.0–16.0 mm and a maximum height of 0.6 mm above the neighbouring rows (usually 0.4 mm or even less). This small and very inconspicuous keel is of a clearly different surface structure from the neighbouring dorsal wrinkles. It is smooth and lacks a crenellated structure. In five specimens the keel was not discernible from the neighbouring dorsal wrinkle rows. There was no analogous medial “wrinkle row” of different keel surface-structure. Although the keel stripe at mid-dorsum is often obvious by its different colour, there is almost no or at least no developed keel structure between the posterior end of the dorsum and the posterior edge of the mantle, contrary to most species of the genus *Tandonia*. One third of adult specimens examined have no keel, which is most uncommon in *Tandonia*. Live and preserved specimens did not differ in the presence/absence of the keel and its structure.

POSTPALLIAL POCKET ORGAN OR WIKTOR'S POCKET ORGAN

On detailed examination of our specimens we found that in some the posterior part of the mantle shrank and became raised due to preservation (BNM 59944). The mantle edge was free and not grown tightly to the dorsum integument. In addition we also detected an unusual postpallial macroscopic structure (Fig. 4C), which is usually hidden under the posterior mantle flap and therefore totally invisible in

live (Fig. 4A, B) and preserved specimens. We noticed the structure because of two slits on either side of the mid-dorsum, just below the posterior free integument flap of the mantle, opening anterior to the beginning of median dorsum wrinkles or to both sides of the keel stripe. Externally it appears to be a paired organ of symmetrical structure and we found it in all specimens of our series of *T. totevi* of all development stages and also in all other species of Milacidae at hand in the BNM collection. The distance between the transverse slits in preserved adult specimens is 3.5–4.5 mm. The slits are $1.5\text{--}2.5 \times 0.4\text{--}1.0$ mm. When the posterior edge of the mantle is lifted the slits are visible macroscopically. For micro-morphological and histological investigations, we selected specimen BNM 59145, a very large specimen, but unfortunately the only specimen in our series in which the structure of the postpallial pocket organ was not disturbed. Since numerous dissections showed that there was no interior structure corresponding to the slits inside the body cavity, we assumed an intra-integumental structure. The observed interior lumen structures turned out to be pockets of varying depth and extension. We found marked differences in the size of the two pockets (Fig. 5). The slit-shaped openings of the left pocket were 1.85 mm transversely and 0.43 mm longitudinally and of the right pocket – 1.35 and 0.25 mm, respectively. The lumen depth was 0.55 mm (left) and 0.2 mm (right). The pockets are surrounded by the tissue of the dorsal body wall and separated anteriorly by a thin septum from the pallial organs. Tubular glands were found only in the anterior walls of the pockets (28 well-defined in the lower third of the left and only one gland near the opening of the right

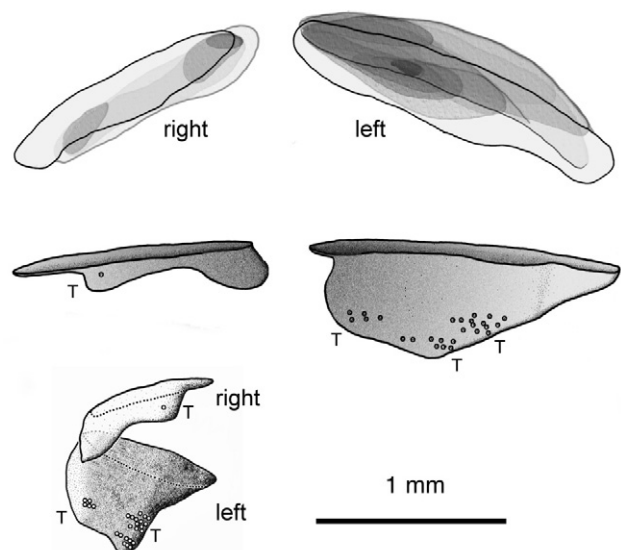


Fig. 5. *Tandonia totevi*, reconstruction of interior dimensions of the postpallial pocket of specimen BNM 59145. Top: dorsal view. Center: dorso-anterior view. Bottom: latero-ventral view. T: tubules; each circle indicates an individual gland as found in the examined specimen

pocket) (Fig. 5, circles). Thick serial sections (10 μm) of the area were initially intended only for three-dimensional reconstruction of Wiktor's pocket organ. Most of the cells of the pocket epithelium were detached and found in the lumen of the pocket. However, in spite of the considerable tissue-shrinkage and autolythic disintegration, important histological details were identified. Locally the 10 μm large cells of cuboid epithelium remained in connection with the subjacent tissue. In the above mentioned areas simple tubular glands were present (Fig. 4D, E). Those extended almost orthogonally to the epithelium about 60 μm into the surrounding tissue and opened into the lumen of the pockets. The 7–8 μm large glandular cells were eosinophilic as found in protein secreting serous glands.

To our knowledge, such a macroscopic structure has never been mentioned in the literature. Therefore we felt free to create a provisional name until its function is studied and explained in detail. The term "postpallial pocket organ" simply derives from its position at the posterior edge of the mantle, the posterior position of shell pocket and especially from its position posterior to the pallial complex and its two transverse pocket-like divisions. We also coined the term "Wiktor's pocket organ" for common future use.

SOLE STRUCTURE

The outermost edge or seam of the sole is well separated from the dorsum by a longitudinal fold, the peripodial wrinkle, which begins left and right of the mouth-flaps and runs posteriorly around the body. The peripodial wrinkle together with the peripodial groove clearly separate the seam of the sole at its outermost posterior end (Figs 2A, B, 3A, B), where the sole is rounded and not pointed. We found this character in live and preserved specimens of all development stages. The structure of the sole itself is typical of Milacidae with two longitudinal grooves and many v-shaped transverse grooves. These grooves cannot be observed in live animals moving over a glass plate, but only in preserved specimens and the visibility depends on the mode of preservation.

INTERNAL STRUCTURE

We dissected specimens stored in 75% ethanol from a few days to 11 months after preservation. We did not find any anatomical differences between the specimens from the Devin and Plovdiv populations, which might be associated with the preservation procedures. Specimens from the valley of Lukovitsa River and "Philippi", Greece, had faded as a result of ethanol preservation. In specimens from all populations the integument was thick and very muscular, especially the mantle, sole and the posterior end of body; the integument of head, nape and anterior sides was

much thinner than other parts. The interior of the integument of specimens from the Plovdiv population varied slightly from light yellow to yellow; the integument of specimens from the Devin population also varied somewhat from light orange to orange. In dissected specimens, regardless of the population, all organs varied only little but an unusual tinge of colour from light yellow to orange was observed, and some parts were glaring brick-red. This tinge of pigmentation faded markedly when ethanol was changed several times during dissection. The digestive gland was of varying colour: from pale orange to light brown. In all specimens the gland extended to the proximal end of the body cavity (Fig. 6A–C). The fibres of connective tissue in all parts were whitish and without any pigmentation.

Genitalia

The data on the genital anatomy derive from fully adult animals dissected at BNM ($n = 10$; Fig. 7A–C):

1. **Gonopore:** it is located at mid body height, near the anterior margin of mantle and is clearly visible in live, extended animals. Its form varies from a vertical slit to a circular or horizontally oval pore, depending on the individual's reproductive condition. A few sexually active animals had a voluminous "papilla" post copulam in the centre of the gonopore (Fig. 1E), which consisted of parts of the anterior portion of the penis (this is not to be confused with the penis papilla inside the posterior portion of the penis). The size of the gonopore is 1.5–5.2 mm vertically and 0.5–3.5 mm horizontally.
2. **Atrium:** The atrium is less than 1 mm in length and characteristically connected to the vagina, the anterior part of the penis and the body wall by a mass of connective tissue and/or very thin muscle fibres. The atrium is whitish, clearly distinguishable from any yellow or orange integument.
3. **Penis:** in all the examined specimens the posterior section resembles a spool as described by WIKTOR (1975). The length and width are similar independent of the total lengths of the specimens. In full length the penis with its anterior and posterior parts is 7.7–8.2 mm. The width of the posterior "spool" is 3.2–3.5 mm. The anterior part is clearly distinct in its form and size and is a short tube only. The walls of the anterior section are very thick and muscular and the lumen is very narrow. The length is 2.4–3.2 mm and the diameter is 2.5–2.7 mm. The interior is smooth but at about half length there is an additional narrowing. The colour of both parts of penis and epiphallus is usually light creamy yellowish or slightly paler. The interior structures of the walls of posterior section of the penis are delicate, regular, longitudinal but short wrinkles. They begin around the penis papilla and stop at about the middle of the anterior part of the "spool". Further anteriorly the internal wall is smooth without

any structure until the fleshy narrowing of the anterior part of the penis. The interior walls around the penis papilla and posterior of it are also completely smooth.

4. Penis papilla: it is located inside the posterior part of penis which is the “spool” and its appearance as described by WIKTOR (1975) is a blunt pointed or almost flat papilla. In some specimens the anterior edge has a distinct but narrow ridge

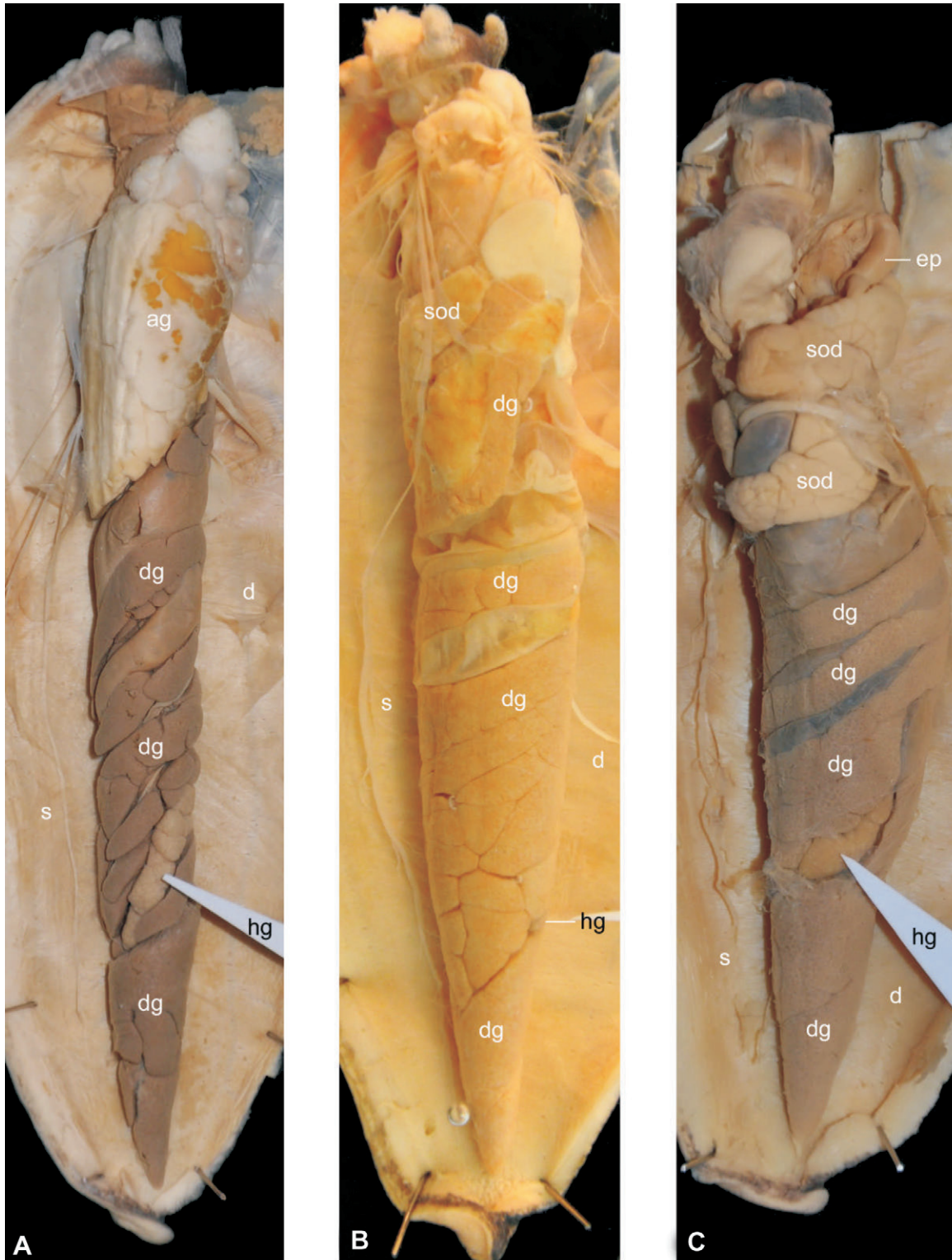


Fig. 6. *Tandonia totevi*, dissections: A – BNM 59526, Plovdiv, Maritza River; B – BNM 59945, Devin Town; C – “Philippi”, Greece. ag – albumen gland; d – dorsum; dg – digestive gland; ep – epiphallus; hg – hermaphroditic gland; s – sole; sod – spermooviduct

or collar anteriorly. Central on top lies the opening of the epiphallus lumen without any special structures. The measurements of papilla penis are 1.9–2.2 mm in width and 1.5–1.7 mm in height.

5. *Musculus retractor penis*: no functional structure like a penis retractor muscle was found. WIKTOR (1975), however, describes the structure as: “very delicate (usually difficult to find at all), in-

serting laterally to anterior section of epiphallus”. We sometimes detected a few rudimentary fibres of connective tissue attached in the area described by WIKTOR (1975) which could be interpreted as remains of a muscle, but these were without any connection to the interior body wall and therefore cannot be described as a retractor muscle. If at all there might be a muscle functioning as a retractor

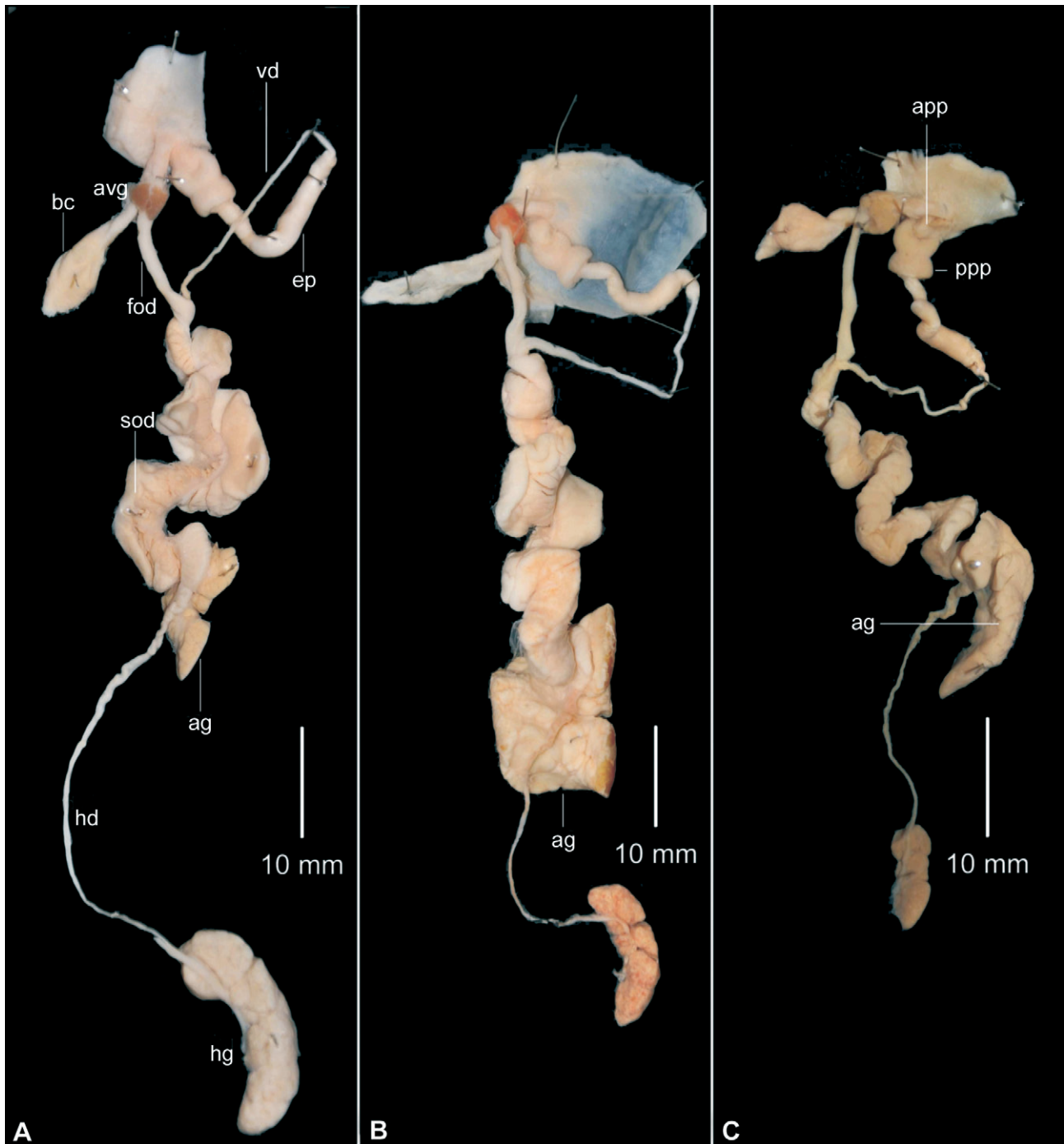


Fig. 7. *Tandonia totevi*, genital organs: A – senile specimen BNM 59145, Plovdiv, Maritza River; B – adult specimen BNM 59946, female stage, topotype; C – specimen “Philippi” from Coll. MAASSEN, Greece, adult male stage. ag – albumen gland; app – anterior portion of penis; bc – bursa copulatrix; avg – accessory vaginal glands; ep – epiphallus; fod – free oviduct; hd – hermaphroditic duct; hg – hermaphroditic gland; ppp – posterior portion of penis; sod – spermoviduct; vd – vas deferens



- muscle, it should be called musculus retractor epiphallus, but we failed to find it in any of the specimens dissected. Histological as well anatomical examination of juvenile and subadult stages in the future should help to resolve this enigma. The situation found might be analogous to that of juvenile and adult specimens of *T. macedonica* (Rähle, 1971) (WIKTOR 1987a).
6. Epiphallus: in all the specimens it is an elongated tube of more or less the same diameter (1.4–2.8 mm), sometimes tapering very little conically at posterior end. It is about 16–20 mm long, which is 2–2.5 times longer than the entire penis and always has its posterior part bent anteriorly. The posterior end is smoothly rounded with vas deferens inserting almost centrally. The walls are thick and muscular, the lumen is narrow with interior longitudinal folds of the same size and a smooth surface.
 7. Vas deferens: it is 18–24 mm long, 0.4–0.6 mm in diameter, whitish creamy, inserting at one end centrally, or almost centrally, into the posterior end of the epiphallus and at its other end into the anterior part of the prostate gland.
 8. Vagina: it is of whitish colour but its surface is covered by a big mass of connective tissue or delicate muscle fibres that also covers the vagina, part of the bursa copulatrix and its duct, the anterior part of the penis and atrium. On dissection this mass of fibres needs to be removed first to get an overview of the structures and details. In diameter the vagina is 1.6–1.8 mm and the length is 5.5–7.0 mm. WIKTOR (1987a) describes it as: “at best as long as half of penis”. We found it to be longer in all adult specimens. The interior structures of the vagina consist of 8–10 longitudinal folds.
 9. Vaginal accessory glands: They are described by WIKTOR (1987a) as: “closely adhering to vagina walls like indistinct grape-like structures or irregular lump”. Probably this description pertains to subadult stage. In our adult specimens we found the glands as a flat, broad, smooth and seamless belt of bright orange to brick-red colour around the vagina without any distinct surface structures.
 10. Bursa copulatrix: it is tightly attached to the spermoviduct by a mass of connective tissue and/or very thin muscle fibres. It is connected to the spermoviduct on its entire length and width. The measurements from insertion into the vagina to the posterior end are 13–15 mm, greatest width 4.5–5.5 mm when empty. It is elongated and more or less tongue-like at its posterior end, sometimes irregular but always tapering into a rounded tip at the posterior end. The anterior part tapers into its duct towards the vagina without a distinct narrowing at the posterior end of the “duct”, like in most other species of the genus. It is of creamy whitish colour, its walls are thin and translucent enough to see its contents shining through. No distinct interior structures were found.
 11. Duct of bursa copulatrix (see also bursa copulatrix): this structure can hardly be called a distinct duct, it is rather the anterior outlet of the bursa copulatrix into the vagina; it is very tightly attached to the vaginal tube. The “duct” inserts to the vagina near or below the covering accessory vaginal glands at a very sharp angle, but the insertion is difficult to find. Its diameter is 1.1–1.3 mm, it is of creamy whitish colour and not translucent, its walls are only slightly thicker than those of the bursa copulatrix. No special interior structures were found.
 12. Free oviduct: it is a creamy whitish, smooth-surfaced and uniform tube-like structure (diameter 1.3–1.4 mm, length 10.5–11.5 mm). No interior structures were investigated.
 13. Oviduct: it is very tightly attached to the prostate gland along its full length. It is creamy white but only discernible with difficulty from the only slightly darker prostate gland. The surface structure is smooth and little lobed.
 14. Prostate gland (see also oviduct): only the first millimetres near the insertion of the vas deferens can be clearly distinguished from the oviduct. The surface is smooth.
 15. Albumen gland: it is always large in the female stage ($14 \times 9.5 \times 6.0$ mm to $30 \times 16.5 \times 8.0$ mm) and lies within the anterior quarter of the body cavity. The colour varies little from whitish creamy to light yellow or pale orange and can partly appear bright lemon yellow (Fig. 6A) which may be an artefact of preservation.
 16. Hermaphroditic duct: its length (38–50 mm) and diameter (0.46–0.61 mm) vary markedly independent of the total length of the specimens. It varies from creamy-white to pale orange and is almost straight in its posterior section and with many narrow coils (19–21) in its anterior section.
 17. Hermaphroditic gland: it is always embedded between the lobes of the digestive gland and sometimes hardly visible. Its colour, position and size vary markedly. The colour ranges from creamy-white to bright orange-brown and light pinkish-brown. The posterior end of the gland is situated 2.0–23.5 mm from the posterior end of the digestive gland. Its measurements are $14.9 \times 5.0 \times 4.2$ mm – $17.8 \times 5.3 \times 6.4$ mm.
- ### Shell
- All ($n = 6$) specimens from the Devin and Plovdiv populations showed surprising features: the inner layer of pocket tissue was covered with a rusty red pigment with small granular encrustations. The vestigial shell, which was tightly attached by muscles at its proximal end, was covered by a layer of this pigment, which built a “dusty” encrustation almost evenly dis-

persed over the shell's surface and could be easily removed with a fine brush. The pigment was also embedded in the calcareous layers of the shell. The form and size were typical of *Tandonia* and did not differ from WIKTOR's (1975: fig. 12) description and illustration. The length was 9.7–11.5 mm, the width 6.0–6.5 mm, the thickness 0.5–1.5 mm. The shell of WIKTOR's paratype was 10.3 mm long and 5.2 mm wide. There were no differences between the specimens from the Devin and Plovdiv populations. All the vestigial shells examined were symmetrical, light rusty coloured but translucent when cleaned from the pigment layer, very thin and fragile and only weakly calcified. Compared to the large size of adult specimens, the vestigial shells were all small.

Radula

The radulae of our series were not examined in detail. Preliminary information for the species was published by WIKTOR (1975).

Jaw

The jaws ($n = 3$) of the slugs from the Devin, Plovdiv and "Philippi" populations were microscopically examined after removing them from the buccal mass. They are typically oxygnath and the colour varies from pale horny yellow to rusty red. The width is 3.9–4.1 mm, the height 2.0–2.2 mm; (2.4×2.2 mm pale horny yellow (Devin); 3.9×2.0 mm pale horny yellow (Plovdiv); 4.1×2.5 mm, rusty red ("Philippi")).

SPERMATOPHORES AND "SPERM PACKAGES"

In nine dissected fully adult specimens we found no spermatophores or their remains in either the bursae copulatrix or in the epiphalli. This is in contrast to what WIKTOR et al. (1994) and WIKTOR (2001) reported for the species on Crete and other islands of southern Greece. Instead of spermatophores in two of the nine specimens dissected (BNM 59945 and 59946) we found a small clump or package (at most $5.0 \times 2.5 \times 1.5$ mm) of a whitish-crumbly mass of ovoid to spherical shape inside the bursa copulatrix; in their shape, colour and texture they resembled the "sperm packages" found in several species of Limacidae after copulation. Microscopic investigations, however, clearly revealed this to be a mass containing cell debris as well as unidentified material and fine tissue fragments of plants, but without spermatozoa. At this stage of research it is impossible to prove the origin of these concretions or aggregations which erroneously had first been suspected to be "sperm-packages". It can be speculated that they derived from a copulation in which the partner did not transfer a spermatophore but only mucus. Plant tissue fragments might have been transported into the organism when adhering to parts of everted genitalia of the partner. In one dissected specimen (BNM 59525) which was found freshly dead upon

arriving at BNM but little damaged by other specimens in the same box, the bursa copulatrix was unusually swollen ($15.5 \times 10.0 \times 6.5$ mm). The organ was totally filled with a clump of material of a pale salmon pink colour, totally different also in consistency from that in the "false sperm-packages". In the specimen examined the bursa copulatrix was ruptured at its posterior end as if damaged from overfilling but the actual cause of death remains unclear. The coagulated clump could be removed in one piece. On dissection we detected surface structures of at least two spermatophore tunicles and finally disentangled five spermatophores, embedded in the whitish mass of unknown origin. All the spermatophores were filled with white spermatozoa clearly visible under magnification, only the anterior and posterior parts of the tunicles were empty. The spermatophores were of similar size, diameter and surface structure.

The spermatophores (Fig. 8A–C) found ($n = 5$) are 40–42 mm in total length and almost bare of spines and spikes. The width of the middle portion varies little (diameter 1.70–2.17 mm). In cross-section they are rounded, tapering slightly to the posterior end of the tunicles' lumen. There, an easily discernible horn-like structure (4.3–4.6 mm) is separated from the lumen of the tunicle. In one case (BNM 59525/A) this structure was very short (0.85 mm) as if

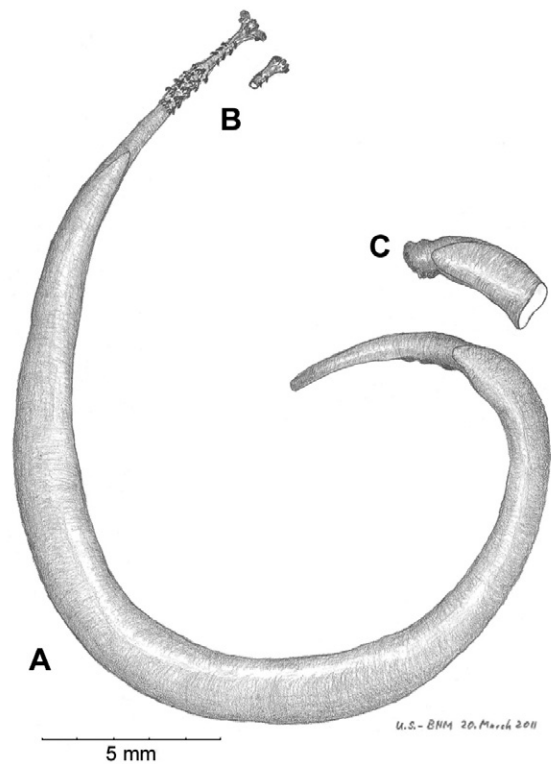


Fig. 8. *Tandonia totevi*, spermatophores found in specimen BNM 59525 from Plovdiv, Maritza River: A – entire spermatophore BNM 59525/B; B – anterior portion of spermatophore BNM 599525/C with short lobes; C – posterior portion of spermatophore BNM 59525/A with short "horn"

misshapen for an unknown reason. The tunicle tapers little anteriorely. Shortly after the anterior end of the lumen of the tunicle there is a short (1.5 mm) and bare narrowing (diameter 0.5 mm), followed anteriorly by a stretch (1.6 mm) of the same diameter which is full of tiny dichotomously four-branched spines. Along the next stretch (1.5 mm), between the four-branched spines and the “head” of the spermatophore, there are only small and simple unbranched spikes or sometimes only a single simply forked spine. The “head” (diameter 0.94 mm) is shaped like a four-lobed crown or blossom and lacks hooks or spines. In one case (BNM 59525/A) the “head” had markedly shorter lobes (diameter 0.60 mm). The spermatophores are of a shiny coppery colour. This colour is the most saturated at the posterior and anterior ends of the spermatophore as well as at the outermost ends of the very tiny spines and spikes. Besides the unexpectedly huge size of these spermatophores, it is surprising that the tunicles’ walls are not only extremely thin and fragile but also absolutely translucent. Spermatozoa masses inside the spermatophores, coagulated because of preservation in ethanol, were easily visible under a small magnification as a white mass inside the tunicles’ lumen.

The white mass surrounding the knot of spermatophores was investigated microscopically. It turned out to almost exclusively consist of spermatozoa but also contained aggregated mucus and rounded cells (Fig. 9A) of unknown origin and function. The overfilled and ruptured bursa, containing five huge spermatophores and additional spermatozoa seems to be an unnatural situation, at least according to what we currently know about Milacidae.

SPERMATOZOA

Small pieces of the whitish masses found in the bursa copulatrix of the slugs BNM 59945 and 59946 were composed of detritus-aggregations without spermatozoa whereas in the slug BNM 59525 they consisted of accumulations of detached cells of unknown origin mingled with spermatozoa (Fig. 9A). It was very difficult to separate intact spermatozoa from the other contents because in most cases the flagella broke off during preparation. Some of them remained unbroken and in such cases the flagella were about 135 μm long (Fig. 9B). It is uncertain, however, if any flagella were completely intact. The spermatozoon head is a helical structure which is usual in Stylommatophora (HEALY 2001) appearing as a flat ridge in *T. totevi*. The head is 11 μm in length and has a spiral apical acrosomal region (Fig. 9C1, D1). Adjacent to the apex the middle part of the head with a diameter of 1.7 μm contains the 7 μm long nucleus (Fig. 9C2, D2). At its base, the site of the axoneme-root, the diameter of the head is 1.4 μm (Fig. 9C3, D3). Methylene blue or toluidine blue stain the nucleus intensely but the apex and basal region of the head have a pale appearance. Probably due to shrinkage of the spermatozoon during preservation, the head is separated in the nucleus-midpiece junction, the neck-region, from the midpiece and only the axoneme bridges the gap of approximately 1.5 μm as a thin filament with a diameter of about 0.15–0.3 μm (Fig. 9C4, D4). The midpiece possesses two helically coiled ribbons arranged in opposite directions, the glycogen helices which are mitochondrial derivatives. This is unusual because in Stylommatophora normally only one gly-

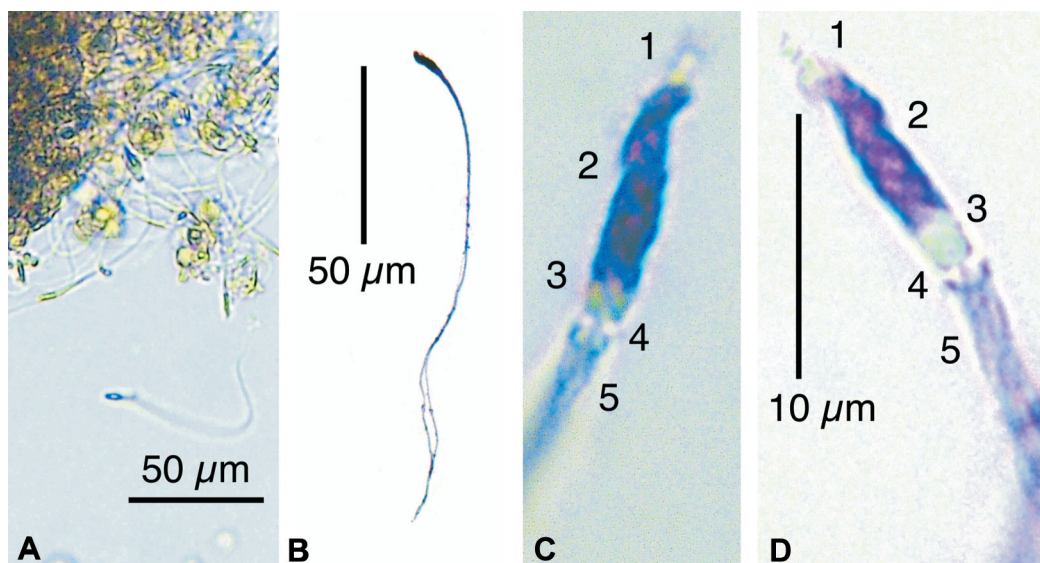


Fig. 9. Spermatozoa found in the bursa copulatrix of *Tandonia totevi* BNM 59525: A – accumulation of detached cells of unknown origin mingled with spermatozoa, unstained; B – two spermatozoa close together, methylene blue; C – spermatozoon head with helical ridge, same magnification as D, methylene blue; D – spermatozoon head with helical ridge, toluidine blue. 1 – pale apex; 2 – nucleus; 3 – pale basal region; 4 – basal part of the axoneme in the neck-region; 5 – midpiece with two glycogen helices arranged in opposite directions

cogen helix is present. In contrast, multiple glycogen helices are found e. g. in Ellobiidae, Vaginulidae, and in Basommatophora. Near the head the midpiece measures about 1.7 μm between the ridges (Fig. 9C5, D5). In all investigated Stylommatophora the mitochondrial derivatives form the terminal portion of the spermatozoon and there is no glycogen piece preceded by an annulus as found in other Gastropoda (HEALY 2001). For this reason the midpiece merges seamless into the tail.

EGGS

The only clutch of eggs found during our investigations was laid by the specimens of our series BNM 59616–59622 at the end of January 2010 (Fig. 10). It consisted of eight eggs only and it was found at the depth of 10–20 mm in the soil of the vivarium. First hatchlings were observed on 22nd February 2010, when starting their surface activity. The empty egg envelopes did not stick together in a cluster but were found separately in the soil (distance 5–10 mm). They were washed under running tap water and preserved in 3% formaldehyde solution (BNM 60083/A-H). They were ovoid and their measurements were 5.5–6.0 \times 5.0–5.5 mm. The envelopes were translucent and not pigmented but with visible white calcium carbonate crystals embedded in them.

MATING BEHAVIOUR

Copulation was first observed by D. GEORGIEV in a plastic container with two specimens (series BNM 59525–59530) from the Plovdiv population of markedly different size and body mass. The slugs had been collected in the field on the 10th November 2009. On the 2nd December 2009 at 06:45 they were observed snuggling in pre-copulation behaviour (Fig. 1C) on a pillow of moss but no iridescent slime trails and no copulation slime patch were visible. Copulation took

place on the 4th December 2009 at night. Unfortunately, exact times were not recorded but the copulation lasted very long, until the next day (Fig. 1D). No movements (encircling) were observed at this stage of copulation because the phase of encircling obviously had already been over and the slugs were motionless. Remains of a short iridescent slime trail were visible for some centimetres before the couple had built a copulation slime patch on the ground. Most probably the phase of following each other prior to copulation was short because of the limited space in the small container. Leaving almost no visible iridescent slime trails the slugs stayed with their soles on the soil on a slime patch of an almost circular form (diameter about 60 mm) which was partly visible during copulation. The bodies of both partners were curved strongly, with the heads to the right. The smaller partner was in full body contact with the larger one and snuggled around the anterior part of its mantle, left side of mantle and anterior left side of the body. The gonopores were in contact and from the everted genitalia only a very small rounded and pale milky white swelling was visible between them (probably slightly everted parts of atria of both partners). During copulation, the heads of both partners were hidden under the anterior part of mantle. The mantles were contracted and anterior flaps directed downwards almost to the ground, the ommatophores and tentacles of both specimens were hidden, the pneumostomes were completely closed. Unfortunately, no further details of copulatory behaviour could be observed in these two animals.

Another mating was observed by U. E. SCHNEPPAT and R. CORNU in a vivarium at BNM with three adult specimens (series BNM 59945–59947) kept together for observation for about 12 months. On the morning of the 15th January 2011 at 07:00 we found numerous iridescent slime trails (on the glass and ground) and an almost circular slime patch (diameter 65 mm) on the ground. Copulation, unfortunately, could not be

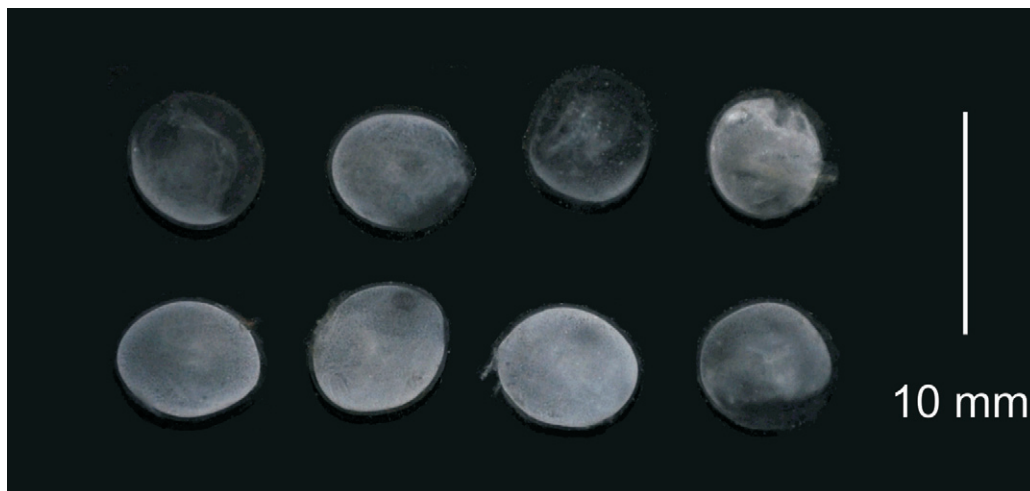


Fig. 10. *Tandonia totevi*: egg envelopes of hatched batch BNM 60083/A–H



observed but from the signs found we concluded that pre-copulation behaviour included intensive head-to-tail following for several metres (in the comparatively small vivarium of 40 × 21 × 25 cm about 190 cm of iridescent slime trail could be identified), then circling and producing an iridescent sticky slime patch on the ground. On this occasion, we observed that one of the animals clung to the vivarium's wall head up and in an almost stretched position (BNM 59946). The specimen did not move and its ommatophores and tentacles were retracted while the head was well visible. The genital pore was still a very swollen circular structure with a central "papilla". The other specimen (BNM 59945) involved in the copulation snuggled to the third slug (BNM 59947) on the ground (Fig. 1E) and its gonopore showed the same characteristic structure of a circular swelling with an interior "papilla" well visible. Dissection of preserved animals proved that this "papilla" was actually a part of the interior tissue from the anterior portion of penis and not to be mistaken with the penis papilla. No spermatophore parts were visible in the gonopores.

REPRODUCTION AND LIFE CYCLE

Our captive animals copulated on the 2nd December 2009 and the 15th January 2010. One cluster of eggs was laid around the end of January (see above). This is the first record of breeding under captive or semi-natural conditions. Copulations have never been observed before. The dates suggest a single phase of reproduction in the winter. Juveniles hatched three to four weeks later. At some stage of growth the captive juveniles matched the size of juveniles collected by WIKTOR at Devin in May as well as those collected by I. DEDOV near Shiroka Laka in the Lukovitsa river valley. It is possible that the small juvenile specimen from Devin collected in December was a slowly growing individual of a late clutch. The size and weight of juveniles hatched in captivity after 12 months were 56–75 mm total length and 3.2–6.0 g. Ideal conditions in the laboratory (no low temperatures, abundant food) may have contributed to accelerated growth in captivity, but this remains a speculation.

From all our observations, we concluded the following stages of reproduction and life cycle:

1. Fully adult animals start pre-copulation behaviour and copulate in late autumn or early winter (02.12.2009 at 12.4°C air temperature in captivity). Climatic conditions at Devin and Plovdiv can be very mild in November and December.
2. Eggs are laid in late winter, probably deep in the soil.
3. Hatching, depending on the temperature of the site, probably takes place from late winter to early spring.
4. The juvenile period lasts up to 20–26 months. Even large specimens collected in Bulgaria were

classified as juveniles due to their closed gonopores.

5. WIKTOR (1975) described a specimen collected at Devin in May as a young adult in early male stage (subadult!) with its gonopore only partly open and the albumen gland not well developed. Development to full maturity takes another 6–8 months (until October–December).
6. It is assumed that the animals die shortly after reproduction and there is no indication of a second reproductive period. In captivity, the slugs can die without obvious reason, even when kept under "ideal" conditions, and then are scavenged by their conspecifics, at least in vivaria. The first signs of an animal dying include remaining motionless and cessation of feeding. The dorsum and posterior body part seem to be paralysed when the animal tries to move. Another indication is loss of weight (35–50%) and shrinking size, even when food is available ad libitum.
7. The maximum life span is probably 2.5–3 years.

FEEDING

Neither fresh faeces nor digestive tract contents of specimens collected in the field have been analysed, but we have observed the species feeding on debris in its natural habitat in Bulgaria.

In captivity the animals were fed on and accepted the fruit flesh of cucumber but not the skin; they ate carrots, flesh of grapes (without skin) and also dry cat food pellets (beef), moist rotting oak leaves (*Quercus* sp.) as well as green algae from dead timber and rotting bark. Fungi, potentially nutritious (*Agaricus bisporus* and *Pleurotus ostreatus*), were offered, but only little was eaten, contrary to most other species of slugs we keep in captivity in the lab at BNM (Arionidae, Milacidae, *Boettgerilla pallens* Simroth, 1912, Limacidae and *Bielzia coerulans* (Bielz, 1851)). It seems that the offered fungi species, and probably fungi in general, are not part of *T. totevi*'s natural diet. Animal tissue, such as raw pork and fish food granules (produced from cattle liver), was not eaten. The wet paper towels lining the boxes and vivaria were always eaten, regardless of other food available. This suggests that cellulose, bacteria and yeasts growing on the paper might be of nutritional value, but also other components of the paper may be concerned. Subalpine forest mosses arranged for shelter in the vivaria were not eaten. Like many other slug species, *T. totevi*, at least in captivity, scavenges on conspecifics.

SOCIAL BEHAVIOUR

We never observed any aggressive behaviour towards conspecifics in captive conditions. We think that the observed bite marks are caused by syntopic *Limax* spp. in Bulgaria (e. g. *L. cf. graecus* at the

Plovdiv site). In captivity *T. totevi* seems peaceful and mostly found in close body contact with its caged conspecifics. Up to seven adults were found “snuggling” together in one vivarium and we never observed specimens hiding singly under shelter.

DISTRIBUTION

When not otherwise stated, locality coordinates and altitudes a. s. l. were reconstructed from Google Earth, Version Google 2011. The distribution is shown in Fig. 11.

In Bulgaria the species is known from three sites, two of them in the Western Rhodope Mountains:

1. Devin town, which is the type locality where the species was first collected by WIKTOR in May 1967 and described in 1975. The species was re-found by A. CHAUSHEV at Devin Town, Nastan city area, in December 2009. Here we give the coordinates and altitude from where the topotypes were collected by A. CHAUSHEV because it was impossible exactly to reconstruct WIKTOR’s collection site of the holotype and paratypes. The type locality is about 21°42'39.30"N and 24°25'08.10"E, 786 m a.s.l. Devin is situated in the Province of Smoljan, Bulgaria.
2. The valley of Lukovitsa River, Turlata Ridge, Province of Smoljan, Bulgaria. The collecting site was

along the country road between Shiroka Laka and Gela and was found by IVAILO K. DEDOV on 4th August 1997. This site is only 16 km from the type locality at Devin.

3. The third site known in Bulgaria is situated in the Upper Thracian Plain, northwest of Plovdiv along the left bank of Maritza River. This site lies north of the Western Rhodope Mountains and was discovered by D. GEORGIEV on 15. September 2009. The site is situated in the Province of Plovdiv. It is 53 km from the type locality.

The only locality reported from Greece is within the distributional range of “true” *T. totevi*. WIKTOR (1987a) mentioned six specimens from the area of ancient ruins at Philippi/Filippoi, Administrative Division of East Macedonia and Thrace, Prefecture of Kavala. For more details see “Material examined...”. W. J. M. MAASSEN (personal communication) collected only one specimen which is still available in his private collection. Five specimens mentioned by WIKTOR (1987a, 2001, WIKTOR et al. 1994) are missing and not traceable. This single specimen available as a voucher is a true *T. totevi* sensu WIKTOR (1975). The locality is 82 km south of the type locality and situated in the southern foothills of the Rhodope Mountains in Greece.

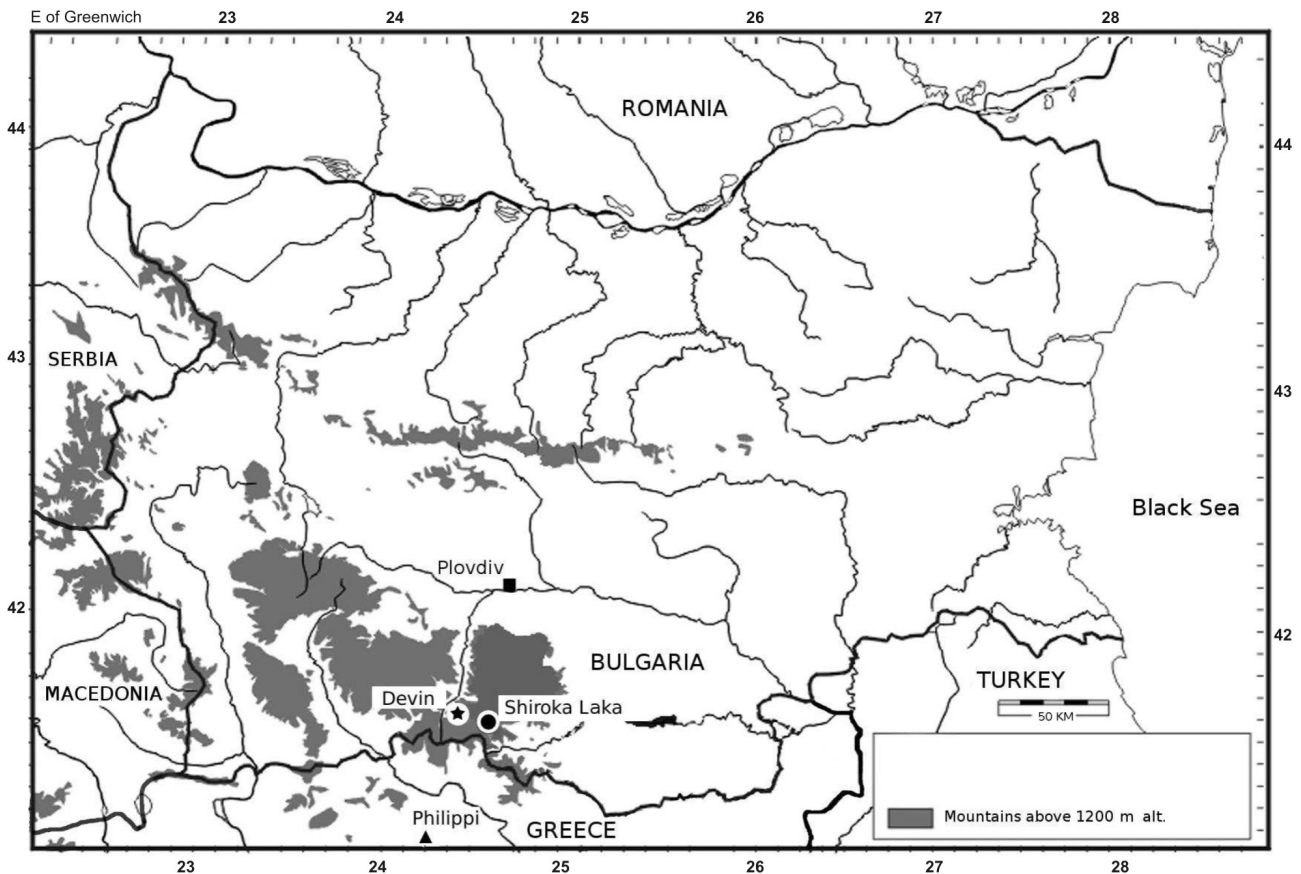


Fig. 11. *Tandonia totevi*, sites in Bulgaria and Greece, 1975–2011: ★ – locus typicus at Devin Town; ▲ – site at “Philippi”, Greece; ■ – site near Plovdiv, Maritza River; ● – site between Shiroka Laka and Gela, Lukovitsa River valley

Samples from other sites in southern Greece (the islands of Crete, Kimolos, Karpathos, Kasos and Kythira) reported by MYLONAS (1982), WIKTOR et al. (2001) and WIKTOR (1994) proved to be a different and undescribed species, which is also the case of the finds from the Peloponnese and the island of Euboea (T. de WINTER personal communication). The taxonomy of these is doubtful and it is likely that at least two more species occur there. The specimens are deposited and documented in the Collection of National Natural History Museum Leiden, The Netherlands. The spermatophore morphology in these populations is markedly different from that of *T. totevi* (WIKTOR 1975) from Bulgaria and northeastern Greece. *T. totevi* was obviously confused with several other species since its first records on the island of Kimolos. The range of the true species is still poorly known. *T. totevi* is known to occur in Bulgaria and in northeastern Greece only.

HABITAT

The habitats in the three Bulgarian sites and in the one in northeastern Greece differ markedly. The localities at Devin, near Plovdiv and near "Philippi" are clearly anthropogenetic. The site in the valley between the villages Shiroka Laka and Gela is in a subalpine forest with meadows:

1. At Devin (locus typicus) sandy soils prevail. The bedrock in the area contains very little calcium and mainly consists of gneisses and andesites. The type locality is described (WIKTOR 1975) as "near gardens by the road...synanthrope". Also at Devin where A. CHAUSHEV re-found the species it was collected in the yard of a house (not very far from the forested slopes!) and also at the porch of a small family house at 786 m a.s.l. Today it is not known if the species also lives in the area of Devin, outside the town in natural habitats and it is therefore too early to state that the species is a strict synanthrope and possibly has been introduced in the area. At this locality *T. totevi* was found to co-occur with *Limacus flavus* Linnaeus, 1758 (WIKTOR 1975) but no information concerning other species of Gastropoda is available.
2. Near Plovdiv the species occurs on sandy soil (mostly quartz and silicates with very little calcium and almost no clay) of the flood plain forest along the Maritza River and a small creek as well in the cracks of a huge wall which is built of granite cubes. The soils of the local area are derived from alluvium of the Maritza River and its tributaries. The second locality in Bulgaria is a restricted and small area inhabited by *T. totevi*. This site is situated on the northern bank of the Maritza River at 162 m a.s.l. A railroad bridge crosses the river and there is a small irrigation canal from the nearby rice fields. Originally this bank was a wild and undisturbed

floodplain forest dominated by *Salix* spp., *Populus* sp., *Sambucus* sp., *Acer negundo*, *Ailanthus altissima*, *Juglans regia*, *Ficus carica*, *Robinia pseudoacacia*, *Amorpha fruticosa*, *Rubus* sp. and *Alnus* sp. but also *Typha* sp., *Urtica dioica*, *Rumex* cf. *longifolius*, *Dactylus glomerata*, *Phragmites australis*, *Humulus lupulus*, *Clematis vitalba* and *Cannabis sativa* occurred there. It is not known whether *T. totevi* has been introduced there by man or if it is a relic of a former and wider distribution in this area. The site is not far from the mouth of the Dermendere River, a southern tributary of the Maritza River. It needs to be proven whether or not the population was established by colonisers brought by the river from the Rhodope Mountains. The species reproduces naturally at this site. Here *T. totevi* lives together with *T. kusceri* (Wagner, 1931), *Limax* cf. *graecus* Simroth, 1889, *Limacus flavus* (Linnaeus, 1758), *Deroceras reticulatum* (O. F. Müller, 1774), *D. turcicum* (Simroth, 1894), *Oxychilus glaber* (Rossmässler, 1835), *Cepaea vindobonensis* (Pfeiffer, 1828), *Xerolenta obvia* (Menke, 1828), *Monacha* sp. and *Helix lucorum* Linnaeus, 1758. The last species is extremely common at this site.

3. In the valley of Lukovitsa River between the villages Shiroka Laka and Gela the bedrock is built of gneisses and andesites with very low calcium content. The site is not only the highest known (1,300 m a.s.l.) but it is also situated in an almost natural subalpine coniferous forest dominated by *Picea abies*. In our opinion *T. totevi* is a natural inhabitant of this site and area. The two juvenile specimens found there on 04. August 1997 indicate that the species reproduces naturally at the site. The following gastropods co-occur with *T. totevi*: *Alinda atanasovi atanasovi* (Urbański, 1964), *Cattania trizona rumelica* (Rossmässler, 1835), *Laciniaria plicata rhodopensis* Nordsieck, 2008 and *Macedonica marginata* (Draparnaud, 1801). Twenty more species of Gastropoda are known from the area and valley between the villages mentioned, among them the following slugs and semislugs: *Arion subfuscus* (Draparnaud, 1805), *Daudebardia wiktoria* Riedel, 1967, *Deroceras turcicum* (Simroth, 1894), *Lehmannia nyctelia* (Bourguignat, 1861), *Limax* cf. *cinereoniger* Wolf, 1803, *Phenacolimax annularis* (Studer, 1820), *Tandonia serbica* (Wagner, 1931) and *T. kusceri* (Wagner, 1931). The search for Gastropoda in this area was carried out by I. K. DEDOV in August 1997. None of the species detected at the site and in the area directly indicates anthropogenetic introductions of gastropods.
4. No detailed information is available on the site at "Philippi", Greece. The altitude is only 54 m a.s.l. It is the lowest-situated and the southernmost record of the species.

All the known localities and potential habitats of *T. totevi* are in need of a thorough ecological research.

POPULATION DENSITY

The populations at Devin and Plovdiv do not seem to be small, even if the population near Plovdiv seems to be restricted to a very small area. We conclude that, since at least 23 specimens have been collected and observed at the same very small site in 2009 and 2010 near Plovdiv, the species cannot be rare at least at this site. Eight specimens at Devin collected by A. WIKTOR and five specimens collected by A. CHAUSHEV indicate the same. The species is strictly nocturnal and its activity might depend on the weather, humidity, temperature, wind, soil structure and density of vegetation. Even though the slug is astonishingly large, its colouration and normally slow movements and also the usually “dry” and non-shiny appearance of its skin make it almost undetectable even on bare ground or on leaf litter (Fig. 1A). From late spring on, when the vegetation is already dense, it probably will be almost impossible to find even a single specimen because of its perfect camouflage.

DISCUSSION

T. totevi was previously regarded as “a species poorly known” (WIKTOR 2001) and rare (IRIKOV & ERÖSS 2008) as well as “endemic to the Western Rhodopes of Bulgaria only” (IRIKOV & MOLLOV 2006). Since its first collection at the type locality at Devin in 1967 (WIKTOR 1975) there were only few records of the species in Bulgaria. We collected all information known about the species from Bulgaria and northeastern Greece; for many years there was almost no research on the slug. At this stage it is much too early to conclude that the species is “rare” (IRIKOV & ERÖSS 2008). The species is not endemic to the Bulgarian Western Rhodopes (IRIKOV & MOLLOV 2006) because of its occurrence at “Philippi”, Greece, published already by WIKTOR (1987a). It is not surprising that a strictly nocturnal slug, hiding deep in the soil and in crevices of rocky slopes during daytime, is difficult to detect without a thorough search at night, during the slugs’ natural activity period. It is therefore advisable that the future mapping of the species should be carried out at night, using torchlight.

Species identification in case of juvenile slugs is difficult because their genitalia are not yet developed. Nevertheless, we compared the external characters of the two voucher specimens (from I. K. DEDOV’s collection) from the valley of Lukovitsa River, which had been dissected previously, with those of eight juvenile specimens of comparable size and development stage from the Plovdiv population, one from the Devin population and also 30 specimens of *T. sowerbyi* from the

CONSERVATION AND THREATS

The site near Plovdiv has changed since the construction of the granite wall and the railway bridge many years ago. More recently, industrial plants, a road and a parking lot were built there. The site is seriously threatened by heavy bulldozers digging for sand and by deforestation authorised by the governmental authorities at Plovdiv. These destructions are carried out under the label of floodplain management. The destruction eradicated almost the whole ecosystem and the mosaic of floodplain habitats of the Maritza River at its northern bank. The destruction of the many slow-running creeks, ponds, wetlands, willow thickets, dry sunny and sandy beaches, etc. and their biodiversity occurred in the winter 2009/2010. The only reason why the site of *T. totevi* was not destroyed totally was the existence of the high granite wall along the river bank. The wall is maintained only to preserve the industrial plant, parking lot and the road above it. The site of *T. totevi* is polluted with garbage, especially plastic and sewage is discharged into the river from the factory.

island of Ischia, Italy. The latter species could be excluded easily because of its consistently fewer groove lines between the slit of the pneumostome and the midline of the dorsum (= 16). In addition, when all the specimens of *T. sowerbyi* were compared with the slugs of the same size found in the valley of Lukovitsa River (50 mm sole length when preserved), they turned out to be fully adult. It must be mentioned that *T. sowerbyi* has not been found in Bulgaria to date. We found no differences between the voucher specimens of *T. totevi* in question and the juveniles from Plovdiv and Devin, and also no affinities at all to other species (e. g. *T. pinteri* (Wiktor, 1975)) of the genus in this area. The latter species has only 11–12 groove lines between the slit of pneumostome and mid-line of dorsum and, when fully adult, is about the same size as the specimens from the Lukovitsa River or even smaller. However, future research will have to verify our identification, especially regarding adult samples from the area between Shiroka Laka and Gela and their genital characteristics.

Thirty six years after the original description it is now clearly possible to distinguish *T. totevi* from other species of the genus of comparable size and pigmentation, by its constant genital characters and the size and structure of its spermatophores. Nevertheless, there are several taxa described which are in urgent need of careful research, comparison, redescription and even description, which still cause problems and confusion. Populations reported by MYLONAS (1982),



WIKTOR et al. (1994) and WIKTOR (2001) from southern Greek islands as *T. totevi* are definitely not conspecific with our specimens because of the many differences in the genital anatomy and different structure and size of the spermatophores. These are in need of redescription and a new name, with a careful revision of the whole group of large *Tandonia* (*T. cretica* (Simroth, 1885), *T. lagostana* (Wagner, 1940)). Also large species of *Tandonia* from the island of Euboea and the Peloponnese externally resemble *T. totevi*. These, however, are clearly different in the structure of their genital apparatus and spermatophores (T. DE WINTER personal communication) and are in need of research and description.

This project shows how little in fact is known about very large species of slugs in Europe. The newly discovered intra-integumental postpallial pocket organ/Wiktor's pocket organ of still unclear function requires further research. The toxins detected in *T. budapestensis* (Hazay, 1881) (SYMONDSON 1997 and personal communication) might be associated with this organ, which we also found to be present in *T. budapestensis*. Unanswered questions concerning the reproduction biology and life cycle as well as distribution of *T. totevi* also require further research.

Many questions still have to be answered concerning the species and many details still remain unknown which will have to be resolved by future students. This indeed will be absolutely necessary not only in the southernmost insular populations of the species in Greece, but also on the island of Euboea and the Peloponnese (T. DE WINTER personal communication), where many of the characters do not fit those of

the type specimens from Bulgaria and north eastern Greece. Additional research is necessary to explain the function of the newly detected organ described here. Although we describe additional characters and add the first information concerning the bionomics of the species in Bulgaria and north eastern Greece extensive research is necessary.

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REFERENCES

- DEYRUP-OLSEN I., LUCHTEL D. L. 1998. Secretion of mucous granules and other membrane bound structures: a look beyond exocytosis. *Int. Rev. Cytol.* 183: 95–141.
- HEALY J. M.: 2001. Spermatogenesis and oogenesis. In: BARKER G. M. (ed.). *The biology of terrestrial molluscs*. CABI Publishing, Oxon, UK, pp. 357–382.
- IRIKOV A., ERÖSS Z. 2008. An updated and annotated checklist of Bulgarian terrestrial gastropod (Mollusca: Gastropoda). *Folia Malacol.* 16: 199–207.
- IRIKOV A., MOLLOV I. 2006. Terrestrial gastropods (Mollusca: Gastropoda) of the Western Rodopes (Bulgaria). In: BERON P. (ed.). *Biodiversity of Bulgaria. 3. Biodiversity of Western Rhodopes (Bulgaria and Greece) I*. Pensoft & Nat. Mus. Natur. Hist., Sofia, pp. 753 - 832.
- LUCHTEL D. L., DEYRUP-OLSEN I. 1991. Ultrastructure and lysis of mucin containing granules in epidermal secretion of the terrestrial slug *Ariolimax columbianus* (Mollusca: Pulmonata). *Cell Tissue Res.* 266: 375–383.
- MYLONAS, M. 1982. The zoogeography and ecology of the terrestrial molluscs of Cyclades. Zoological Laboratory and Museum University of Athens, Ph. D. Thesis.
- NITZ B., HEIM R., SCHNEPPAT U. E., HYMAN I., HASZPRUNAR G. 2009. Towards a new standard in slug species descriptions: The case of *Limax sarnensis* Heim & Nitz n. sp. (Pulmonata: Limacidae) from the Western Central Alps. *J. Moll. Stud.* 75: 279–294.
- SYMONDSON W. O. C. 1997. Does *Tandonia budapestensis* (Mollusca: Pulmonata) contain toxins? Evidence from feeding trials with the slug predator *Pterostichus melanarius* (Coleoptera: Carabidae). *J. Moll. Stud.* 63: 541–545.
- WIKTOR A. 1975. New slug species (Pulmonata: Milacidae and Limacidae) from the Balkan Peninsula. *Ann. Zool.* 33: 1–15.
- WIKTOR A. 1981. Genus-group level classification of Milacidae. *Malak. Abh.* 7: 145–153.
- WIKTOR A. 1983. The slugs of Bulgaria (Arionidae, Milacidae, Limacidae, Agriolimacidae – Gastropoda, Stylomatophora). *Ann. Zool.* 37: 71–206.
- WIKTOR A. 1986. Slug distribution in Greece. *Proceedings of the Eighth International Malacological Congress UM'83, (Budapest, Hungary) 28. August – 4. September 1983.*



- Hungarian Natural History Museum, Budapest, Hungary: 295–300.
- WIKTOR A. 1987a. Milacidae (Gastropoda, Pulmonata) – systematic monograph. *Ann. Zool.* 41: 153–319.
- WIKTOR A. 1987b. Spermatophores in Milacidae and their significance for classification (Gastropoda, Pulmonata). *Malak. Abh.* 12: 85–100.
- WIKTOR A. 1997. Endemism of slugs within the Balkan Peninsula and adjacent islands (Gastropoda: Pulmonata: Arionidae, Milacidae, Limacidae, Agriolimacidae). *Genus* 8: 205–221.
- WIKTOR A. 2000. Agriolimacidae (Gastropoda: Pulmonata) – a systematic monograph. *Ann. Zool.* 49: 347–590.
- WIKTOR A. 2001. The slugs of Greece (Arionidae, Milacidae, Limacidae, Agriolimacidae, Gastropoda Stylommatophora). *Fauna Graeciae* 8. Natural History Museum of Crete, Hellenic Zoological Society, Irakleio.
- WIKTOR A., VARDINOYANNIS K., MYLONAS M. 1994. Slugs of the Greek Southern Aegean Islands (Gastropoda terrestria nuda: Milacidae, Agriolimacidae et Limacidae). *Malak. Abh.* 17: 1–35.

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