

***Enchytraeus andrasiformis* Nagy, Dózsa-Farkas et Felföldi, 2023 (Oligochaeta Enchytraeidae) from *Caretta caretta* (Linnaeus, 1758) (Testudines Cheloniidae) nests in Tuscany, Italy**

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ABSTRACT

Invertebrates infesting sea turtle nests are rarely identified to species. Here we report on the enchytraeid oligochaete worm *Enchytraeus andrasiformis* Nagy, Dózsa-Farkas et Felföldi, 2023 from a nest of the loggerhead sea turtle, *Caretta caretta* (Linnaeus, 1758) (Testudines Cheloniidae), on a beach in Tuscany, Italy. *Enchytraeus andrasiformis* belongs to the *Enchytraeus albidus* species complex, a taxonomically difficult group of closely related species that live as decomposers in organically enriched habitats of the marine littoral. Detailed morphological investigations and DNA sequencing of the COI barcode gene was carried out to assure a correct identification. The species diagnosis is revised and the biogeography of species of the *E. albidus* complex in the Mediterranean Sea is discussed. We hypothesize that the worms of *E. andrasiformis* were attracted to the nest by previously damaged and decaying eggs.

KEY WORDS

Enchytraeus; infestation; Clitellata; sea turtle eggs; Tuscany; Italy.

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INTRODUCTION

Loggerhead sea turtles - *Caretta caretta* (Linnaeus, 1758) (Testudines Cheloniidae) - lay eggs in nests on sandy beaches. Survival, growth and development of the embryos are threatened by multiple factors, among them predators and parasites. Nests with high mortality of eggs or embryos are often infested with invertebrates of different taxa, such as Acari, Nematoda, larvae of Diptera, Coleoptera, or Neuroptera, and also Oligochaeta

(Andrews et al., 2016). However, it is difficult to establish whether these invertebrates are the causal agent of lower hatching success, as predators or parasites, or whether they are simply attracted, as scavengers, by otherwise impaired nests due to the higher amount of decaying organic matter (Andrews et al., 2016). Unfortunately, the invertebrates are rarely identified below family level (e.g., Aymak et al., 2020). Regarding oligochaetes, they have been identified as belonging to Enchytraeidae in nests on Mediterranean beaches in Turkey (Aymak et al.,

2017, 2020) but left unidentified in nests in Cyprus (Broderick & Hancock, 1997) and Turkey (Katilimis et al., 2006; Urhan et al., 2010; Baran et al., 2021). Infestation was often correlated with elevated egg mortality (Baran et al., 2001; Özdemir et al., 2006; Urhan et al., 2010).

In summer 2021 a massive presence of oligochaetes was discovered in a nest in Marina di Grosseto, on the west coast of Italy. This is the first report of an infestation of sea turtle nests in Tuscany. The discovery was made in the framework of protection activities for sea turtles, carried out by the NGO tartAmare (www.tartamare.org). This NGO monitors the nesting activities of *Caretta caretta* on beaches in Marina di Grosseto in order to increase hatching success of this species in spite of strong pressure by tourism facilities (Fig. 1). Nests are observed, protected, and relocated, if necessary. Among the seven nests monitored during the nesting season of 2021, two of them suffered from high mortality, correlated with repeated inundation (see below). In one of them oligochaetes were found.

In this paper, the oligochaete species is described and identified as a member of the species complex around *Enchytraeus albidus* Henle, 1837 (Enchytraeidae). Parts of this complex have recently been resolved with combined methods of DNA sequencing and morphological investigation (Erséus et al., 2019), and new species of this group are currently being discovered (Arslan et al., 2018; Nagy et al., 2023; Torii et al., 2023). However, morphological species identification is difficult because of minute differences among species and unknown intraspecific variation ranges of taxonomically important characters. Therefore, a detailed description of specimens is recommendable, together with DNA sequencing, to achieve accurate identification results.

The specimens of *Enchytraeus* found in the sea turtle nests were first tentatively identified as *Enchytraeus irregularis* Nielsen et Christensen, 1961, but further scrutiny showed that they actually belong to a species recently described from Tuscany, *E. andrasiformis* Nagy, Dózsa-Farkas et Felföldi, 2023. Here we provide a description of the specimens,

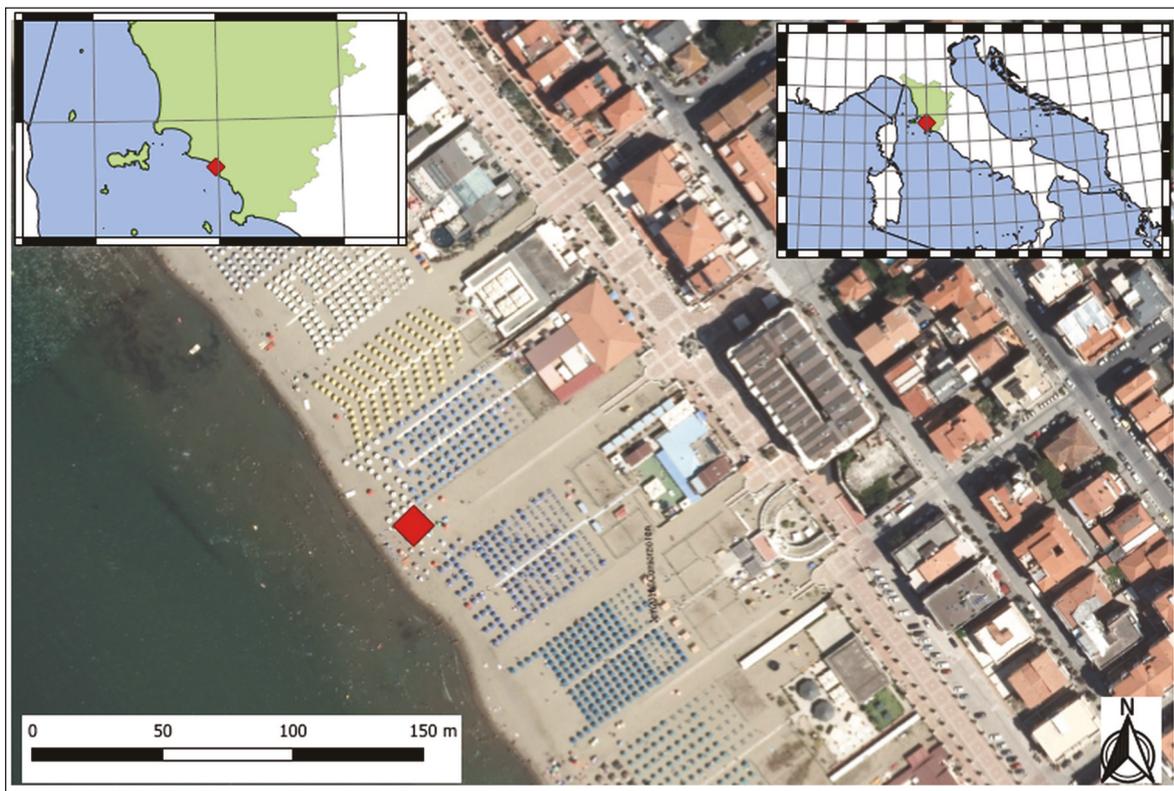


Figure 1. Airview and geographical location of the *Caretta caretta* nests in Marina di Grosseto, Italy.

together with a DNA barcode of one of them, and we discuss the possible role of this invertebrate in the reduced hatching success of sea turtles.

MATERIAL AND METHODS

The site is located in the southern part of Tuscany, Italy, at Marina di Grosseto (42°43'1.16"N, 10°58'45.54"E), on a beach with low slope (Fig. 2, Table 1) and a high density of deck chairs in the tourist season. The sand is granulometrically highly uniform (Cu close to 1); the respective parameters are given in Table 1.

On the night of 21 July 2021 a loggerhead sea turtle was observed digging her nest near a

bathroom (Fig. 2) close to the shoreline. On the morning of 22 July the nest was re-located by LP on the same beach at 27 m distance from the shoreline (Fig. 2). To monitor temperature, an iButton thermometric sensor was placed closed to the nest and at the same depth of the egg chambers. The nest was protected with a fine-meshed gauze of 2x2 m. On 25 July and 19 September 2021 the nest was flooded by sea tide water for several hours (Fig. 2). Ambient temperature at the latter date dropped temporarily from 26.5 °C to 24 °C. The nest was inspected on 20 September 2021. The chamber had fungi and numerous white oligochaetes, presumably enchytraeids, on the eggs and in the adjacent sandy interstices (Fig. 2). Oligochaetes were collected and preserved in



Figure 2. Locality of enchytraeid worms and site history. From left to right and top to bottom: Sea turtle depositing eggs below deck chairs, 21.VII.2021. - Relocated nest. - Nest inundation 25.VII.2021. - Nest inundation 19.IX.2021. - Same as before. - Nest inspected 20.IX.2021.

ethanol 95% and 70%, respectively, and sent to RMS for microscopical study.

For microscopical investigation, five specimens, one of them preserved in 95% ethanol, were passed through an ethanol-xylol dehydration series and mounted whole in Malinol, a synthetic substitute for Canada Balsam. They were then investigated with a compound microscope equipped with interference contrast (Nomarski) optics, digital camera for photography, and a drawing tube. The specimen preserved in 95% ethanol was cut in half prior to microscopical

Parameter	Value
D10	0.13 mm
D60	0.2 mm
Cu (D60/D10)	1.53
L*	27 m
Dh	0.63 m
Slope (Dh/L)	2.33%

Table 1. Granulometric and other physical site data. D10: Particle size below which are 10% (by weight) of sediment. D60: Particle size below with are 60%. Cu: Uniformity coefficient, adimensional. L: Horizontal distance to the sea in m. Dh: Vertical distance to the water table of the sea in m.

investigation, and the posterior end was sent to the lab of MJK for DNA sequencing. The specimen was subadult and its internal DNA lab specimen code is CE39992.

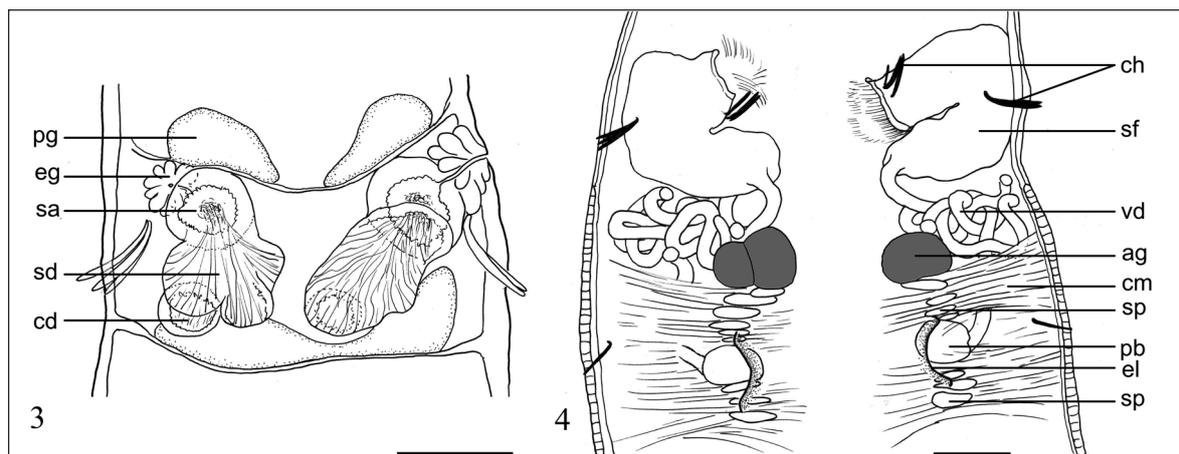
DNA was extracted from the amputated posterior end, using QuickExtract DNA Extraction Solution 1.0 (Epicentre, Madison, WI, USA). From the extract two genetic markers (COI and ITS2) were amplified using PCR, following primers and programs found in Martinsson et al. (2017: Table S2). Sequencing was carried out by MWG Eurofins Operon (Edersberg, Germany), sequences were assembled in Genious 6.1.8 (<https://www.geneious.com>, Kearse et al., 2012), and deposited in Genbank, accession numbers OR416420.1 (COI) and PP084620 (ITS2). The five specimens are deposited in the Museo Civico di Zoologia, Roma (Italy).

RESULTS

Enchytraeus andrasiformis Nagy, Dózsa-Farkas et Felföldi, 2023 - (Figs. 3–12)

Enchytraeus andrasiformis Nagy, Dózsa-Farkas et Felföldi, 2023: 107-145, Figs. 1E, 5.

Enchytraeus irregularis Nielsen et Christensen, 1961. Schmelz et al., 2022.

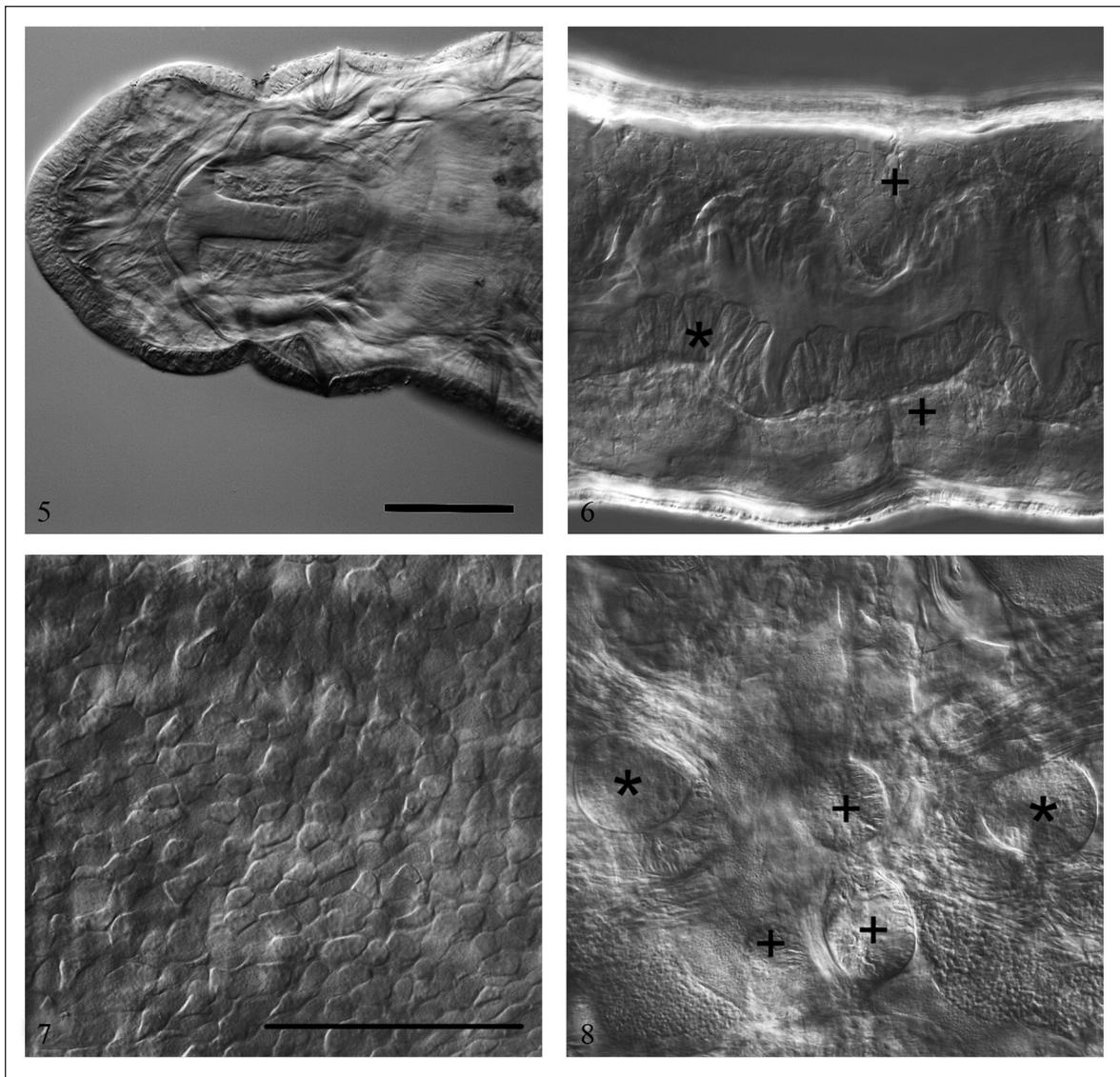


Figures 3, 4. *Enchytraeus andrasiformis* Nagy, Dózsa-Farkas & Felföldi, 2023, line drawings. Fig. 3: spermathecal region, dorsal view. Fig. 4: clitellar region, ventral view. ag: accessory gland; cd: blind-ending continuation of diverticulum, oriented ventrad; ch: chaetae of XI; cm: copulatory body muscles; eg: spermathecal ectal gland; el: epidermal lip overhanging male pore; pb: primary glandular bulb of male copulatory organ; pg: pharyngeal gland of IV, dorsal lobe; sa: spermathecal ampulla; sd: spermathecal diverticulum; sf: sperm funnel; sp: secondary papilla; vd: vas deferens. Scale bar: 100 μ m.

MATERIAL EXAMINED. Four adult specimens, preserved in 70% ethanol, whole-mounted unstained in Malinol. One subadult specimen (CE39992), preserved in 95% ethanol, anterior end (23 segments; posterior end used for DNA extraction).

DESCRIPTION. Body length 7–9.5 mm, diameter at V 0.33–0.43 mm, at XII 0.37–0.65 mm, at XX 0.4–0.6 mm. Body tapering anteriorly and

posteriorly. Segment number 35–40. Chaetae (Figs 3, 4, 5, 10, 11) three in most bundles, also two, rarely four, arranged in regular fan. Chaetal formula $(2)3 - 2, \underline{3} : 3 - (2)3(4)$. Two chaetae often in lateral and ventral bundles of XIII–XV. Up to six in bundles where chaetae are being replaced or old chaetae have not been shed completely, here arrangement irregular. Chaetae present laterally in XII, 2 per bundle, small (Fig. 4). All chaetae of



Figures 5–8. *Enchytraeus andrasiformis*, photographs from whole-mounted specimens. Fig. 5: anterior end, dorsal view, with prostomium, anterior bifurcation of dorsal blood vessel and lateral chaetae of II, III. Fig. 6: midbody region, lateral view, with midgut pars tumida (star), and thick coverage of chloragocytes (+). Fig. 7: clitellum, dorsal view. Fig. 8: ventral view of XII, specimen with three accessory glands (+), one of them out of focusing plane. Stars: Primary male bulbs. Scale bars: 100 μ m, one for 5–6, one for 7–8.

same shape, simple-pointed, straight in ectal 2/3, tapering towards both ends, bent hook-like entally. Largest chaetae 80–85(–97) μm long and 6–7 μm thick, smallest chaetae c. 50 μm long and 4 μm thick. Caudal chaetae not enlarged.

Prostomium shorter than wide (1:2–1:3, preparation artefact) (Fig. 5), head pore not seen. Presence of epidermal gland cells not evident, epidermis rugose in one specimen. Body wall c. 20–30 μm thick, longitudinal muscle layer about as thick as layer of epidermis plus ring muscles. Cuticle thin, <1 μm thick, inconspicuous. Brain c. 120–150 μm long, c. 1.5x as long as wide, rounded, tapering anteriorly. Oesophageal appendages paired, short, unbranched, with wide lumen, c. 5–6x as long as wide, length c. 160–180 μm , diameter c. 30 μm , simple elongate sacs extending into IV, opening jointly in oesophagus mid-dorsally in III behind pharyngeal pad. Pharyngeal glands (Figs 5, 10–12) with dorsal and ventral lobes, dorsal lobes largest in IV, smallest in VI, connected or separate in IV, V, and VI. Chloragocytes (Fig. 6) vesicular, higher than wide, dense layer surrounding gut, filling most of coelom, diameter 14–18 μm apically, height 35–50 μm . Dorsal blood vessel with prostomial bifurcation (Fig. 5), posterior origin not distinguished. Midgut pars tumida in XXV–1/2XXVII (subadult specimen, 35 segments), ventral, elevated to different degrees, with interspaces, cells granular (Fig. 6). Coelomocytes with conspicuous spherical granules of equal size; size of cells not measurable due to coagulation.

Clitellum from XII–XIII, saddle-shaped, behind female pores 2–7 midventrally continuous rows of gland cells. Diameter of gland cells 8–14 μm , height laterally 18–22 μm ; pattern reticulate or in indifferent rows (Fig. 7). Testes and sperm funnels in XI, ovaries, vasa deferentia and male pores in XII. Sperm sacs paired in X–XI or IX–XI, large, filling coelom almost completely. Mature spermatozoa aligned on top of sperm funnel; length of sperm heads not measured. Sperm funnels compressed, difficult to measure, about 300 μm long and 3x as long as wide, collar c. 100–120 μm wide (Fig. 4). Vasa deferentia coiled in XII, apparently short, not extending beyond clitellum, diameter 18–21 μm , not conspicuously enlarged in mid-region (Fig. 4). Male efferent apparatus (Figs 4, 8, 9) with primary glandular bulb, diameter 100–110 μm , attached to end of vas deferens, and

anteriorly and posteriorly a longitudinal row of secondary glandular papillae, intervened by copulatory muscles. Bursae proper or a copulatory field apparently absent, but each male pore in a depression of the body surface, overhung by a lip the size of the primary bulb, projecting medially (Fig. 4). One, two or three accessory glands present in male pore region in 3 out of 5 specimens, anteriorly, between, or behind male pores, position symmetric or asymmetric; compact, rounded bodies, attached to inner side of body wall, not larger than primary male gland (except in the single subadult specimen, here accessory glands larger), diameter 65–85 μm (specimen with 3 accessory glands) or 100–120 μm (specimen with 1 accessory gland) (Figs 4, 8, 9). Subneural glands absent. Spermathecal ectal duct completely glandular (Figs. 3, 10, 11), c. 90 μm long, less than 1.5x as long as wide; ampulla (Figs. 3, 11, 12) wider than ectal duct, thick-walled, with one large dorsal diverticulum of varying and irregular shape, more than twice as long as wide, larger than ampulla, smooth or with infolds or outfolds, thin-walled entally, with heads of spermatozoa attached to inner wall (Figs. 3, 10–12). Separate openings of ampullae into lateral sides of oesophagus, by means of a short ental duct.

DNA SEQUENCES. A fragment of the mitochondrial Cox I gene was successfully sequenced, with a length of 658 base pairs, from the subadult individual of our series, specimen ID CE39992, GenBank accession number OR416420.1. Also a 429 bp fragment of the nuclear ribosomal Internal Transcribed Spacer 2 (ITS2) was obtained from the same specimen, GenBank accession number PP084620. COI showed a 97% match with the COI sequence fragment of a specimen from the type series of *E. andrasiformis* (specimen ID. P.145.1, Genbank accession number MZ750823). ITS showed a 100% match with the ITS fragment of the same specimen.

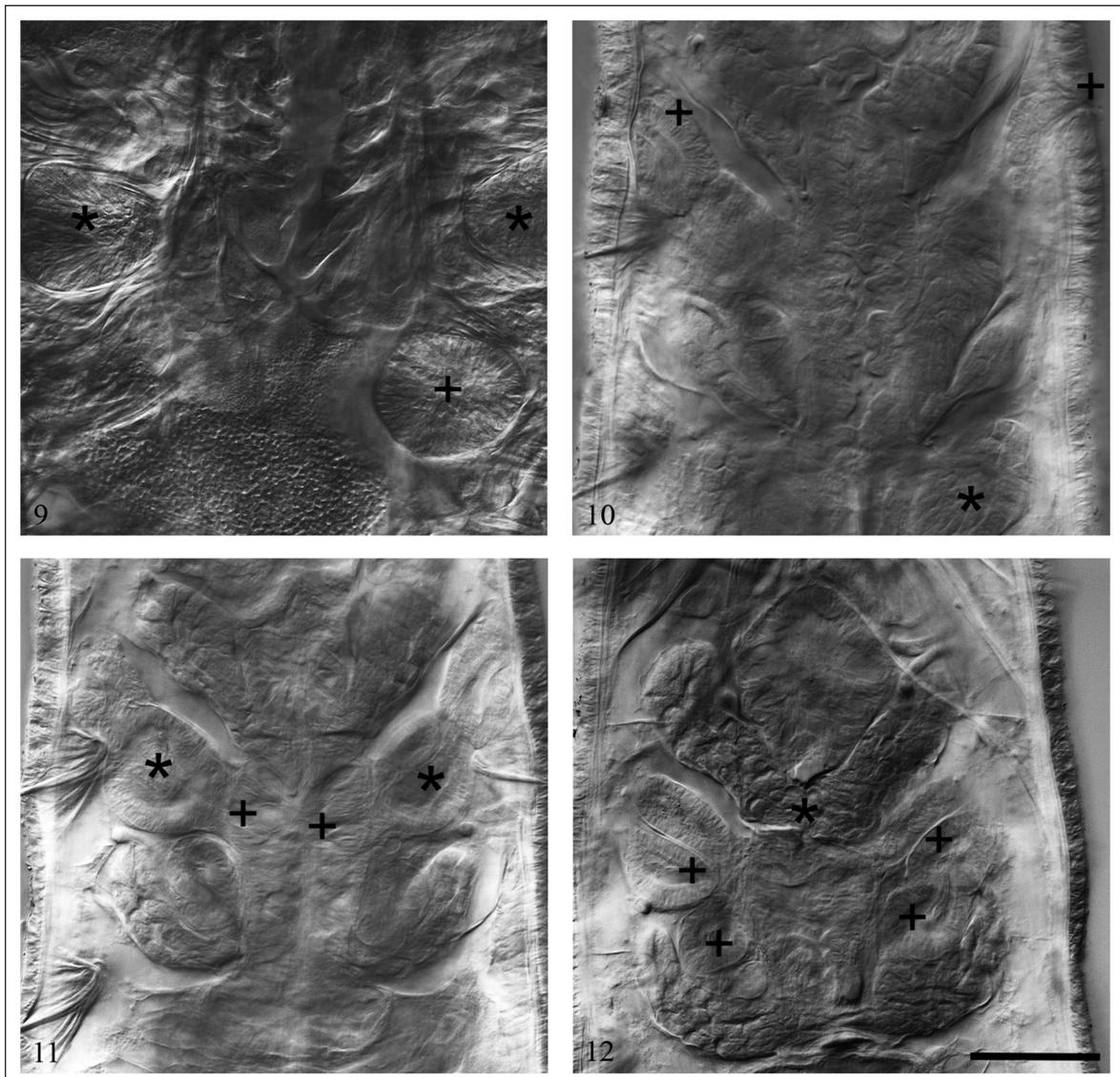
DISCUSSION

The Enchytraeus albidus species group

As regards the identity of the species, the investigated specimens belong to a species related to the *Enchytraeus albidus* group. This group is distinguished within the genus, following Schmelz

& Collado (2010), by short and tube-like oesophageal appendages and a male reproductive system that, apart from a central male glandular bulb, often includes a number of smaller secondary papillae around the male pores. Many species of this group are comparatively large (length >10mm, up to 30 mm) with high segment number (>40, usually >50) and a maximum number of up to 4 or

5 chaetae in at least some regular bundles, but there are also species in the size range of our specimens (Schmelz et al., 2019). Species of this mainly holarctic group live in the marine upper littoral, in brackish water, and in organically enriched habitats such as compost heaps. An exceptional habitat is the profundal of the highly alkaline Lake Van in eastern Turkey, where *E. polatdemiri* Arslan et



Figures 9–12. *Enchytraeus andrasiformis*, photographs from whole-mounted specimens. Fig. 9: ventral view of XII, specimen with one accessory gland (+). Stars: Primary male bulbs. Figs. 10–12: dorsal view of IV–VI with pharyngeal glands and spermathecae, same object in slightly different focusing planes. Fig. 10: lateral chaetae V, VI visible to the left. Spermathecal ectal pore focused to the right (+), spermathecal ectal duct with glands focused to the left (+). Star: Dorsal pharyngeal gland lobe in VI. Fig. 11: spermathecal ampullae (stars), lateral connection with oesophagus (+). Fig. 12: dorsal diverticulum, variously folded on both sides (+). Dorsal connection of pharyngeal glands in IV (star). All photos oriented with anterior end to the top. Scale bar: 100 μ m, the same for all.

Timm, 2018 is among the very few invertebrate species to tolerate such extreme conditions (Arslan et al., 2018).

In a molecular genetic analysis (Erséus et al., 2019), *Enchytraeus albidus sensu Auctorum* was recognized as an assemblage of species, three of which received formal names, *E. albidus* Henle, 1837 *sensu stricto*, *E. moebii* Michaelsen, 1885 (revalidated after decades of synonymy with *E. albidus*), and *E. albellus* Klinth, Erséus et Rota, 2019 in Erséus et al. (2019); the identification of a fourth clade was tentative (as *E. cf. krumbachi* Čejka, 1913). Recently, Nagy et al. (2023) and Torii et al. (2023) added descriptions and DNA sequences of further species of the *E. albidus* group: five species, three of them new to science, in Nagy et al. (2023): *E. adrianensis* Nagy, Dózsa-Farkas et Felföldi, 2023, *E. andrasi* Nagy, Dózsa-Farkas et Felföldi, 2023, and *E. andrasiformis* Nagy, Dózsa-Farkas et Felföldi, 2023. The other two species in that paper are *E. irregularis* Nielsen et Christensen, 1961 and *E. krumbachi*, the latter with confirmed species identity (see above, Erséus et al., 2019). Torii et al. (2023) described *E. ohtakai* Torii, Akagi, Uchino & Kobayashi, 2023 from sewage beds of a plum processing plant in Japan. To summarize, there are now 10 nominal species of the *E. albidus* group with morphological descriptions and DNA sequences. Further species in this group, not yet characterized by DNA sequences, are *E. kincaidi* Eisen, 1904, *E. mediterraneus* Michaelsen, 1926, and a number of other species placed in synonymy with *E. albidus* by various authors but which may be revalidated (like *E. moebii* Michaelsen, 1885) when DNA sequences become available.

A suggested monophyly of this group within the genus *Enchytraeus* (Erséus et al., 2019) found molecular (DNA sequence-based) support in Nagy et al. (2023) only when the newly included species *E. andrasi* and *E. andrasiformis* were excluded: these two species appeared more closely related with species of the so-called *E. buchholzi*-group as defined in Schmelz & Collado (2010). This would mean that the *E. albidus* group, defined morphologically (as in Schmelz & Collado, 2010), is not monophyletic, or that this group, defined as a monophylum that includes *E. albidus* s. str. (as in Erséus et al., 2019), cannot be defined

morphologically. In the following, we use the morphological concept of this species group.

Differences within the E. albidus group

Nagy et al. (2023) provide a very detailed comparison of species in the *E. albidus* group, excepting *E. ohtakai* and a few others. The species of the *E. albidus* group of which DNA sequences are available can be subdivided into two groups according to body size, maximum number of chaetae and extension of the vasa deferentia. Five species have large body (length > 15 mm), a maximum of more than 3 chaetae per bundle, and vasa deferentia that extend beyond the clitellum: *E. albidus* s.str., *E. moebii*, *E. adrianensis*, *E. albellus*, and *E. krumbachi*. Among these, the latter three have a distinct dilatation of the vasa deferentia in the mid-section, a character rare in enchytraeids. The other five species are smaller (body length < 15 mm), they have a maximum of 3 chaetae per bundle, and vasa deferentia do not extend beyond the clitellum: *E. polatdemiri*, *E. andrasi*, *E. irregularis*, *E. andrasiformis*, *E. ohtakai*. It is this group that has three species with “extra” accessory male glands: *E. irregularis*, *E. andrasiformis*, and *E. ohtakai*. Within *Enchytraeus*, only these three species have accessory male glands.

Taxonomic identity of the collected specimens

The collected specimens were first identified tentatively as *E. irregularis* Nielsen et Christensen, 1961 (Schmelz et al., 2022), because it was, at that time, the only species in the *E. albidus*-group with accessory glands in the male region. *E. irregularis* had been redescribed by Hong & Dózsa-Farkas (2018). Further common features were: chaetal pattern (mostly 3 per bundle, not regularly 4 or 5), segment number (< 50), and posterior extension of vas deferens (confined to XII, not extending into XIV or following segments). However, the identification was tentative because of two peculiarities of *E. irregularis* that differ from our specimens: (1) The spermathecal ectal duct is covered with gland cells only near the ectal pore, while the ental part leading to the ampulla is free of gland cells; (2) accessory glands were found so far in all specimens, and their distribution is always pairwise, either in front of the male pore, or behind,

or in both positions (Nielsen & Christensen, 1961; Hong & Dózsa-Farkas, 2018).

Later, descriptions of two further species with accessory glands were published almost simultaneously, *E. ohtakai* Tokii, 2023 from a sewage plant in Japan, and *E. andrasiformis* Nagy, Dózsa-Farkas et Felföldi, 2023, from Tuscan beaches close to our sea turtle monitoring sites. Our specimens fit better with the descriptions of these two species than with *E. irregularis*: in both species the spermathecal ectal duct is completely covered with gland cells; furthermore, there are variations in the accessory glands similar to those observed in our specimens: in *E. andrasiformis* they are present or absent; in *E. ohtakai* they are in variable positions, either pairwise ventro-laterally or unpaired mid-ventrally.

The collecting locality of the type series of *E. andrasiformis* in Castiglione della Pescaia is not more than 6 km away from the sea turtle nest locality, practically on the same beach. The geographical proximity and the 97% identity of the COI DNA sequence fragment (specimen CE39992) with the one from the type series of *E. andrasiformis* (specimen P141.5) prove that both sets of specimens belong to the same species, hence *E. andrasiformis* is the correct name of the species found in the sea turtle nest. On the other hand, similarity of the COI fragment of our specimens and those collected in Japan is only 82%, indicating that the latter is indeed a different species, despite its morphological similarity.

This description confirms most of the features as given in the original description, above all the variability of accessory glands with respect to position and presence/absence. It seems to differ with respect to the clitellum, originally described as girdle-shaped but saddle-shaped in our specimens, but it may also be conceived as a mixture of both: the region between the bursal folds is always free of glands; before and behind there may be some ventrally continuous rows, but their number is small (2 to 7 transverse rows were counted), and the cells are inconspicuous and much flatter than the lateral and dorsal gland cells. Our description increases the variation range of some characters: segment number goes up to 40 (originally 38), chaetae are up to 97 µm long (up to 87 µm originally), pharyngeal glands may be dorsally separate or connected in IV, V, and VI (originally: separate

dorsally in IV, V, connected in VI), and there are up to 3 accessory glands (up to 2 originally).

Combining both accounts, we present the following revised diagnosis of *E. andrasiformis*, taking into account only characters that are variable in the *E. albidus* group (for a more extended diagnosis see Nagy et al., 2023): Body length ca. 13–20 mm (in vivo), segment number 30–40; chaetae mostly 3 per bundle, 2 often near clitellum, surplus chaetae often present; clitellum saddle-shaped, a few rows continuous mid-ventrally, with flat cells; mid-dorsal connection of pharyngeal glands variable; dorsal blood vessel from XIV, blood colourless; seminal vesicle large; sperm funnel 4–7 times longer than wide, collar as wide as or wider than funnel body; vasa deferentia not extending beyond clitellum, with uniform diameter, no conspicuous dilatations; primary male bulb distinctly larger than adjacent papillae, diameter about 100 µm; accessory male glands present or absent, as large as or larger than primary bulbs; spermathecae with one large and elongate dorsal diverticulum, ectal duct completely covered with ectal glands, ampulla spherical.

Species comparison

Most similar to *E. andrasiformis* is *E. ohtakai*, published almost simultaneously with *E. andrasiformis*. Both species are extremely similar. The following differences of *E. ohtakai* can be extracted from a comparison of the descriptions: Segment number 42–49 (vs. 30–40 in *E. andrasiformis*); dorsal blood vessel origin in XII or XIII (vs. XIV); blood faintly red (vs. pale); male primary bulb not larger than secondary papillae (vs. distinctly larger), distinctly smaller than accessory glands (vs. about as large or larger); accessory glands always present, 2 or 3 in number (vs. 0–3 in number). Of these, the relative size of the primary bulb is possibly the most distinctive or reliable character. The other features may show to be more variable in both species when more specimens have been investigated. Interestingly, there is another species pair in the *E. albidus* group, *E. albidus* s. str. and *E. albellus*, where the relative size of the male primary gland is the only distinctively distinguishing morphological character: about as large as the secondary papillae in *E. albidus*, much larger in *E. albellus*.

Furthermore, *E. andrasiformis* owes its name to its high morphological similarity with *E. andrasi*, a species without accessory glands, and also described as new in Nagy et al. (2023). Again the size of the primary bulb marks a difference: 50–70 µm in maximum length (and thus slightly larger than most of the secondary papillae) in *E. andrasi*, and 80–120 µm (and thus distinctly larger than the papillae) in *E. andrasiformis*. Further differences are slight: coelomocytes are smaller in *E. andrasi*, the sperm funnel is slightly smaller, and its collar is narrower than the funnel body. The two species are said to differ also considering the middorsal connection of the pharyngeal glands (Nagy et al. 2023). This could not be confirmed due to variations of this characters in our specimens (see above). Both species were found in the same area.

To conclude, it seems that the male glandular apparatus is one more key character for the correct morphological identification of species in the *E. albidus* group, apart from other characters such as maximum number of chaetae, size, shape and extension of sperm funnel and vas deferens, and details of the spermatheca. However, with the detection of further species within this species group, morphological distances may become even narrower among species, and DNA sequences will gain even more importance for a correct identification and separation of species.

Species identity of oligochaetes in sea turtle nests

This is the first time that the Oligochaeta infesting sea turtle nests have been identified to the genus and species level. The species identity in the other records is unknown. Aymak et al. (2017, 2020) identified the Oligochaeta worms as “Enchytraeidae”, apparently by their whitish body colour (Aymak et al. 2017, Fig. 13). Enchytraeids are common in organically enriched sediments on the marine shoreline (Giere & Pfannkuche, 1982); the larger forms with more than 15 mm body length and diameter up to 1 mm belong to the genera *Lumbricillus* Ørsted, 1844 and *Enchytraeus*. *Lumbricillus* specimens have rose to red body colour, while *Enchytraeus* spp. are whitish. Therefore the specimens illustrated in Aymak et al. (2017, Fig. 13) probably belong to *Enchytraeus*. The species identity, however, cannot be inferred. Large whitish coastal marine enchytraeids belong

to the *Enchytraeus albidus* group, the diversity of which in the Mediterranean is only very recently being uncovered. Interestingly, none of the Mediterranean specimens investigated by Nagy et al. (2023) belong to *E. albidus* s. str. or to other species common on the northern European shorelines (*E. albellus*, *E. moebii*, see Erséus et al., 2019). Specimens from the Ligurian Sea belonged to *E. andrasi*, *E. andrasiformis* and to *E. krumbachi*, and specimens from Croatia belonged to *E. adrianaensis*. A further Mediterranean species, *E. mediterraneus* Michaelsen, 1926 from beaches in Tunisia, was never recorded again. And two specimens sampled on the Greek island Skopelos do not belong to any species of the *E. albidus* group of which molecular data are currently available (Erséus et al. 2019; Nagy et al., 2023). (The sexually immature specimens were left unidentified but probably belong to a species new to science.) Hence, a picture emerges of not only a north / south division of *E. albidus*-group species, but also of a regional differentiation of species within the Mediterranean basin.

In consequence, large whitish enchytraeids infesting sea turtle nests in different geographic regions of the Mediterranean will probably belong to different species of the *Enchytraeus albidus* group. We hypothesize that the species of enchytraeids to be found in sea turtle nests will depend on the local species pool of free-living *E. albidus* group species. The first example is *E. andrasiformis*: This species was originally described from decaying *Zostera* debris on the shoreline, in close vicinity to our study site (Nagy et al., 2023).

We further expect a much wider distribution of the *E. albidus* group in the Mediterranean than is currently known. Up to now, the sandy marine littoral of the Mediterranean Sea remains practically unsampled for enchytraeids. In fact, we found only two records of “*E. albidus*” in this region, both from studies focusing on freshwater fauna: one record is from brackishwater in the River Gildiz delta in Turkey (Balik et al., 2004) and one from an organically enriched site 7 m asl in Ghazaouet, Algeria (Gagneur et al., 1982).

All previously published reports of sea turtle nests with presence of Oligochaeta worms are from the Eastern Mediterranean, from beaches in Greece, Turkey and Cyprus (Fig. 13) (Andrews et al., 2016;

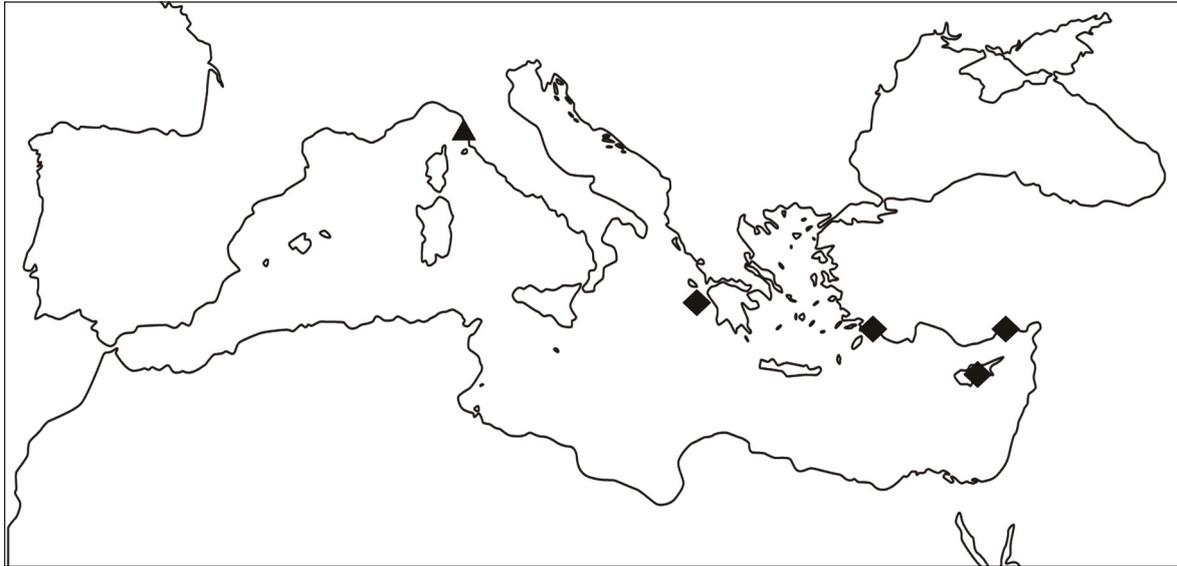


Figure 13. Location of records of *Enchytraeus* spp. in Mediterranean sea turtle nests. Rhombus: previous records. Triangle: Our record.

Aymak et al., 2017, 2020; Baran et al., 2001, 2015, Broderick et al., 1997, Katılmış et al., 2006; Özdemir et al., 2004, 2006; Türkozan et al., 1996; Urhan et al., 2010). Very little is known about the marine littoral enchytraeid fauna in this region. We expect that different, probably undescribed, species, of the *E. albidus* group infested these nests of sea turtles. The present record (Fig. 13, triangle) is the first one from Italy and has a more northern and more western location than the previous findings.

Are enchytraeids a threat to sea turtles?

The final question of our record of *E. andrasiformis* is the role of these worms in the sea turtle nests: Are they a causal agent of lower hatching success, as predators or parasites, or are they attracted, as scavengers or decomposers, by previously impaired nests, due to the high amount of decaying organic matter (Andrews et al., 2016)? We favour the latter explanation, based on two observations: (1) the nest with enchytraeids was among those that had been flooded twice - 25 July and 29 September 2021 - after their relocation on 21 July 2021, together with a significant drop in temperature. These two incidents likely impaired the viability of eggs and embryos. (2) There are no published records of enchytraeids as predators or parasites. A functional shift to a new lifestyle should

be recognizable anatomically, however, *E. andrasiformis*, or species of the *E. albidus* group in general, share the general anatomy of the organ of food uptake common to all enchytraeids and to most other oligochaetes as well: an eversible cushion-like thickening of the dorsal roof of the pharynx, termed pharyngeal pad, invested heavily with musculature and connected with glands that produce the digestive enzymes (Schmelz, 2003). This pad is adapted for uptake of dead or decaying organic matter but not for damaging healthy tissues. It remains possible that enchytraeids enhance a previous damage and thus contribute to the deterioration of the nest. Gajda et al. (2017) have shown that *E. albidus* worms consume within five days a freshly dried leaf of *Sambucus nigra* submerged in water, which suggests the release of exo-enzymes during food uptake, apart from the damaging effect of the suctioning activity of the pad. However, leaves were dried and therefore already mechanically fractioned.

Our interpretation of oligochaetes in sea turtle nests finds support in a study by Hall & Parmenter (2008) who found that the main factor to predispose sea turtle nests to infestation by dipteran larvae was the number of dead eggs and dead hatchlings. The scavenging activity of dipteran larvae was considered beneficial for the beach ecosystem and not a threat to the viability of the sea turtle

populations. The same may be hypothesized for the enchytraeids. In general, enchytraeids are considered primary or secondary decomposers in the soil food web (Potapov et al., 2022), and this is most likely also the function of these worms in the sea turtle nests.

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REFERENCES

- Andrews A., Smith A.C. & Rees A.I.F., Margaritoulis D. 2016. The effect of invertebrate infestation and its correlation with loggerhead sea turtle (*Caretta caretta*) nest success in Laganas Bay, Zakynthos, Greece. *Marine Turtle Newsletter*, 151: 9–15.
- Arslan N., Timm T., Rojo V., Vizcaíno A. & Schmelz R.M., 2018. A new species of *Enchytraeus* (Enchytraeidae, Oligochaeta) from the profundal of Lake Van, the world's largest soda Lake (Turkey, East Anatolia). *Zootaxa*, 4382: 367–380.
<https://doi.org/10.11646/zootaxa.4382.2.8>
- Aymak C., Ergene R.S., Katilmiş Y., Uçar A.H., 2017. Invertebrate infestation in green turtle (*Chelonia mydas* (Linnaeus, 1758)) and loggerhead turtle (*Caretta caretta* (Linnaeus, 1758)) nests on Alata Beach, Mersin, Turkey. *Turkish Journal of Zoology*, 41: 753–761.
<https://doi.org/10.3906/zoo-1509-26>
- Aymak C., Uçar A.H., Katilmiş Y., Babkale E. & Ergene S., 2020. The effect of invertebrate infestation on green turtle (*Chelonia mydas*) nests in Kazanlı Beach, Mersin, Turkey. *Russian Journal of Herpetology*, 27: 245–256.
<https://doi.org/10.30906/1026-2296-2020-27-5-245-256>
- Balik S., Ustaglu M.R. & Yildiz S., 2004. Oligochaeta and Aphanoneura (Annelida) fauna of the Gediz Delta (Menemen-Izmir). *Turkish Journal of Zoology*, 28: 183–197.
<https://journals.tubitak.gov.tr/zoology/vol28/iss3/1>
- Baran İ., Özdemir A., Ilgaz Ç. & Türkozan O., 2001. Impact of some invertebrates on eggs and hatchlings of the Loggerhead Turtle, *Caretta caretta*, in Turkey. *Zoology in the Middle East*, 24: 9–17.
<https://doi.org/10.1080/09397140.2001.10637881>
- Broderick A.C. & Hancock E.G., 1997. Insect infestation of Mediterranean marine turtle eggs. *Herpetological Review*, 28: 190–191.
- Čejka B., 1913. *Litorea krumbachi* n. spec. n. gen. - Ein Beitrag zur Systematik der Enchytraeiden. *Zoologischer Anzeiger*, 17: 145–151.
- Eisen, G., 1904. Enchytraeidae of the West Coast of North America. Harriman Alaska Series, New York, 12: 1–126.
- Erséus C., Klinth M.J., Rota E., De Wit P., Gustafsson D.R. & Martinsson S., 2019. The popular model annelid *Enchytraeus albidus* is only one species in a complex of seashore white worms (Clitellata, Enchytraeidae). *Organisms Diversity & Evolution*, 19: 105–133.
<https://doi.org/10.1007/s13127-019-00402-6>
- Gajda L., Gorgon S. & Urbisz A.Z., 2017. Food preferences of enchytraeids. *Pedobiologia*, 63: 19–36.
- Giere O. & Pfannkuche O., 1982. Biology and ecology of marine Oligochaeta, a review. *Oceanography and Marine Biology Annual Reviews*, 20: 173–308.
- Hall S.C.B. & Parmenter C.J., 2008. Necrotic egg and hatchling remains are key factors attracting dipterans to sea turtle (*Caretta caretta*, *Chelonia mydas*, *Natator depressus*) nests in Central Queensland, Australia. *Copeia*, 2008: 75–81.
- Henle F.G.J., 1837. Ueber *Enchytraeus*, eine neue Anneliden-Gattung. Müllers Archiv für Anatomie, Physiologie und wissenschaftliche Medizin, Berlin, 1837: 74–90.
- Hong Y. & Dózsa-Farkas K., 2018. New descriptions of enchytraeid species (Clitellata: Enchytraeidae) from Korea. *Journal of Species Research*, 7: 80–91.
<https://doi.org/10.12651/JSr.2018.7.1.080>
- Martinsson S., Dózsa-Farkas K., Rota E. & Erséus C. 2017. Placing the forgotten: on the positions of *Euenchytraeus* and *Chamaedrillus* in an updated enchytraeid phylogeny (Clitellata: Enchytraeidae). *Invertebrate Systematics*, 31: 85–90.
<https://doi.org/10.1071/IS16042>
- Michaelsen W., 1886. Über *Enchytraeus möbii* Mich. und andere Enchytraeiden. Lipsius & Tischer, Kiel, 52 pp.
- Michaelsen W., 1926. Zur Kenntnis einheimischer und ausländischer Oligochäten. *Zoologische Jahrbücher. Abteilung für Systematik, Geographie und Biologie der Tiere*, 51: 255–328.
- Nagy H., Dózsa-Farkas K. & Felföldi T., 2023. New insights into the *Enchytraeus albidus* complex (Annelida, Enchytraeidae), with the description of three new species from seashores in Italy and Croatia. *European Journal of Taxonomy*, 870: 107–145.
<https://doi.org/10.5852/ejt.2023.870.2123>
- Nielsen C.O. & Christensen B., 1961. The Enchytraeidae. Critical revision and taxonomy of European species. Supplement 1. *Natura Jutlandica*, 10: 1–23.

- Özdemir A., Ilgaz C., Kumlutas Y. & Durmus S.H., 2006. Invertebrate infestation of *Caretta caretta* nests at Fethiye beaches, Turkey. *Pakistan Journal of Biological Sciences*, 9: 507–513.
- Özdemir A., Türkozan A.O., Ilgaz Ç. & Martin R., 2004. Nest site factors and invertebrate infestation of loggerhead turtle nests. *Israel Journal of Zoology*, 50: 333–340.
<https://doi.org/10.1560/H2RH-M0LA-QKU1-EYV2>
- Potapov A.M., Beaulieu F., Birkhofer K., Bluhm S.L., Degtyarev M.I., Devetter M., Goncharov A.A., Gongalsky K.B., Klarner B. & Korobushkin D.I., 2022. Feeding habits and multifunctional classification of soil-associated consumers from protists to vertebrates. *Biological Reviews*, 97: 1057–1117.
<https://doi.org/10.1111/brv.12832>
- Schmelz R.M., 2003. Taxonomy of *Fridericia* (*Oligochaeta*, *Enchytraeidae*). Revision of species with morphological and biochemical methods. *Abhandlungen des Naturwissenschaftlichen Vereins in Hamburg (Neue Folge)*, 38: 415 + 73 Figs.
- Schmelz R.M. & Collado R., 2010. A guide to European terrestrial and freshwater species of *Enchytraeidae* (*Oligochaeta*). *Soil Organisms*, 82: 1–176.
- Schmelz R.M., Klinth M.J., Chalkia C., Anastasiadou P. & Vavoulidou E., 2019. *Enchytraeus demutatus* sp. nov. (*Enchytraeidae*, *Oligochaeta*) has characters hitherto unrecorded in the genus. *Soil Organisms*, 91: 87–93.
- Schmelz R.M., Pagli D., Scaravelli D., Tagliaferro L., Papetti L. & Klinth M., 2022. Potworms (*Enchytraeus*, *Enchytraeidae*) in a Loggerhead sea turtle (*Caretta caretta*) nest in Tuscany, Italy. Poster at the 7th Mediterranean Conference on Marine Turtles, Tetouan, Morocco.
- Torii T., Akagi T., Uchino T. & Kobayashi T., 2023. A new species of *Enchytraeus* (*Enchytraeidae*, *Clitellata*) from sewage sludge of a plum processing plant in Japan. *Zootaxa* (in press).
- Urhan R., Katılmış Y. & Yüksel M., 2010. Invertebrate infestation in loggerhead turtle (*Caretta caretta*) nests, in Dalyan, Turkey. *Munis Entomology and Zoology Journal*, 5. suppl.: 982–985.

