

First Report of *Aphanurus stossichii* (Digenea: Hemiuridae) from *Engraulis encrasicolus* on the Turkish Coast of the Black Sea, with Light and Scanning Electron Microscopic Observations

Aphanurus stossichii'nin (Digenea: Hemiuridae) Karadeniz'in Türkiye Kıyılarındaki *Engraulis encrasicolus*'tan Işık ve Taramalı Elektron Mikroskopik Gözlemleriyle İlk Raporu

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ABSTRACT

Objective: The aim of this study is to investigate in detail the morphological features of the digenean parasite *Aphanurus stossichii* isolated from the European anchovy *Engraulis encrasicolus* using light and scanning electron microscopy (SEM).

Methods: The specimens of *A. stossichii* were obtained from the pharynx and stomach of the European *E. encrasicolus* caught by commercial fishing vessels in the Black Sea. Parasites were killed in a hot normal saline solution, preserved in to 70% ethanol for light microscopic (LM) studies, and in 2.5% glutaraldehyde for SEM. The morphological diagnostic features of *A. stossichii* were studied in detail under both LM and SEM.

Results: The morphological characteristics of the examined adult *A. stossichii* specimens were found to be similar to the original descriptions describing the basic characteristics of forebody and hindbody shape, the position and shape of the vitellarium, ovary, and testes, and the shapes of oral and ventral sucker. The measurement data for all morphological diagnostics were provided; photomicrographs of each part of the parasite were presented. Infection prevalence, mean intensity and mean abundance values were 8.89%, 4.5 and 0.4, respectively.

Conclusion: All available records of *A. stossichii* morphology are based on light microscope, this is the first study to identification the morphological features of the parasite with SEM. This research is the first on *A. stossichii* presence in *E. encrasicolus* on the Turkish Black Sea coast.

Keywords: Digenea, Hemiuridae, *Aphanurus stossichii*, *Engraulis encrasicolus*, Black Sea, Türkiye

ÖZ

Amaç: Bu çalışmanın amacı, Avrupa hamsisinden *Engraulis encrasicolus* izole edilen digenean parazit, *Aphanurus stossichii*'nin morfolojik özelliklerini ışık ve taramalı elektron mikroskobu (SEM) kullanarak detaylı olarak araştırmaktır.

Yöntemler: *A. stossichii* bireyleri, Karadeniz'de ticari balıkçı gemileri tarafından yakalanan Avrupa hamsisi, *E. encrasicolus*'un farens ve midesinden elde edildi. Parazitler, sıcak tuzlu su çözeltisi içinde öldürüldü, ışık mikroskopik (LM) çalışmaları için %70 etanol içinde ve SEM için %2,5 glutaraldehit içinde muhafaza edildi. Parazitin yüzey anatomisinin morfolojik özellikleri ışık ve SEM kullanılarak detaylı olarak incelendi.

Bulgular: İncelenen ergin *A. stossichii* bireylerinin morfolojik özelliklerinin, ön ve arka vücut şeklinin temel karakterlerini, vitellarium, yumurtalık ve testislerin pozisyonu ile şeklini ve oral ile ventral emici şekillerini tanımlayan orijinal açıklamalara benzer olduğu bulundu. Tüm morfolojik teşhislerin ölçüm verileri sağlandı; parazitlerin her bir bölümünün fotomikrografları sunuldu. Enfeksiyon prevalansı, ortalama yoğunluk ve ortalama bolluk değerleri sırasıyla %8,89, 4,5 ve 0,4 idi.



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Sonuç: *A. stossichii* morfolojisinin mevcut tüm kayıtları ışık mikroskopuna dayanmaktadır. Bu çalışma, bu parazitin morfolojik özelliklerini SEM ile tanımlayan ilk çalışmaya; ve Türkiye'nin Karadeniz kıyılarındaki *E. encrasicolus*'ta *A. stossichii* varlığı üzerine yapılan ilk araştırmadır.

Anahtar Kelimeler: Digenea, Hemiuridae, *Aphanurus stossichii*, *Engraulis encrasicolus*, Karadeniz, Türkiye

INTRODUCTION

The European anchovy, *Engraulis encrasicolus* is one of the most important commercial fish of the Black Sea (1). There are some parasitological studies that have focused on its nematode parasites (2-4). On the other hand, there is no published study so far on the digenean parasites of *E. encrasicolus* from the Turkish coast of the Black Sea. Thus, more studies are needed to determine the parasite fauna of *E. encrasicolus*.

Aphanurus Looss, 1907 is a small hemiurid genus which is represented by 15 nominal species: *A. bailloni*, *A. balticus*, *A. caesionis*, *A. dorosomatis*, *A. dussumierii*, *A. harengulae*, *A. magniprotesticus*, *A. microrchis*, *A. mugilus*, *A. multiprostatatus*, *A. orientalis*, *Aphanurus stossichii* (*A. stossichii*), *A. tuberculatus*, *A. virgula* and *A. xiamenensis*. *Aphanurus stossichii* appears to be a frequently encountered digenean with a wide host range, its main host is probably clupeoid fishes as sardines, sprats, anchovies and sparid as *Boops boops* (5-10). It has been recorded in *Boops boops* living in the Mediterranean and the Aegean Sea in Turkish waters (11,12). In the present study, the specimens of *Aphanurus stossichii* were obtained for the first time from *E. encrasicolus* and the Black Sea coasts of Turkey.

The aim of the present study is to provide light and ultrastructural observations by light (LM) and scanning electron microscope (SEM) of a digenean parasite, *A. stossichii* in European anchovy which was collected from the Sinop coasts of the Black Sea. At the same time, the first detailed observation supplied about this parasite species with this study in Turkey.

METHODS

Sampling of Parasites

Anchovy samples were collected a period from September 2017 to December 2017 from local fishermen in Sinop, Turkey. A total of 45 anchovies were examined for digenean parasites using conventional methods in the parasitology laboratories at Sinop University Faculty of Fisheries. The specimens of *A. stossichii* were obtained from the pharynx and stomach of the European anchovy, *Engraulis encrasicolus* (13). Parasite specimens were set out in a Petri dish containing physiological saline and washed, and counted. Infection prevalence (%), mean intensity and abundance were calculated to according to Bush et al. (13). Morphological diagnostic features of digenean specimens were studied in detail under light and scanning electron microscopes (hereinafter LM and SEM, respectively). Parasite specimens were studied when they were alive and later fixed and preserved in 70% ethanol and Trump's fixative.

Sample Preparation for Light Microscopic Study

Parasite specimens were studied in both alive and permanent preparations. Alive individuals were placed between slide and cover glass without pressure. For permanent preparations, the parasites were killed in a hot normal saline solution, fixed in 70% ethanol and they were stained in acetocarmine stained,

and then examined under an Olympus BX51 microscope and photographed with a DP-25 digital camera. The identification and the morphological characteristics of the parasite were done on the basis of Kostadinova et al. (12) and Gibson (14).

Sample Preparation for the Scanning Electron Microscopic Study

For SEM study, specimens were preserved in a Trump's fixative solution. Samples were placed in 1% osmium tetroxide (OsO₄) in cacodylate buffer for 3 hours and then dehydrated in graded ethanol series (50%, 60%, 70%, 80%, 90%, 95% and 99%). Specimens were dried in an E3100 critical point dryer (Quorum Technologies) using liquid carbon dioxide. Specimens were mounted attached on stubs with double-sided adhesive tape, sputter-coated with a thin layer of gold/palladium Polaron SC-500 and viewed by SEM (Jeol JSM-6510 LV FE-SEM).

Illustrations and Measurements

For light microscopical examination, photomicrography of digenean parasite was taken using a phase contrast Olympus microscope (BX53) equipped with a digital camera (DP50) and all measurements were carried out using an ocular micrometer. Parasitological investigation and LM observations were conducted at the Faculty of Fisheries in Sinop, while specimens for SEM observations were performed at the Kastomonu University, Central Research Laboratory.

Statistical Analysis

No statistical analysis was carried out in this study.

RESULTS

A. stossichii (Monticelli, 1891) Looss, 1907 was detected in the pharynx and stomach of the European anchovy, *Engraulis encrasicolus* (Linnaeus, 1758) in the present study. Infection prevalence, mean density and mean abundance values of *A. stossichii* were determined as 8.89%, 1.5 and 0.13%, respectively.

Taxonomy

Phylum: Platyhelminthes

Class: Trematoda

Subclass: Digenea

Order: Plagiorchiida

Family: Hemiuridae Looss, 1899

Subfamily: Aphanurinae Skrjabin & Guschanskaja, 1954

Genus: *Aphanurus* Looss, 1907

Species: *A. stossichii* (Monticelli, 1891) Looss, 1907

Identifying Characters

Light Microscopy Observations

Body plump, subcylindrical, tapered anteriorly, widest at the level of gonads or vitellarium. Ecsoma absent. The forebody is noticeably shorter than hindbody (Figures 1, 2). The tegument is thick, plicated dorsally along the entire length of body (Figures 3, 4). The ventral sucker is muscular, spherical and larger than

the oral sucker (Figure 2). The oral sucker is spherical and subterminal (Figure 5). Prepharynx absent. Pharynx elongate-oval to subglobular (Figure 5). Oesophagus absent. Sinus sac tubular, narrow. Hermaphroditic duct tubular, its distal half is eversible, forming a temporary sinus organ. Genital pore median, at mid-level of or immediately posterior to oral sucker (Figure 5). Testes are sub-globular or transverse-oval, oblique, contiguous and slightly anterior to mid-hindbody (Figures 1, 6). Seminal vesicle large, elongate-oval, thick-walled, just anterior to or overlapping anterior testis (Figures 1, 2 and 6). Ovary post-testicular, contiguous with posterior testis, transversely oval (Figures 1, 6). Vitellarium is a single large, compact mass, posterior to and contiguous with the ovary, in the third quarter of hindbody (Figures 1, 2 and 7). Eggs are operculate and thin-shelled (Figure 7). Measurements are given in Table 1.

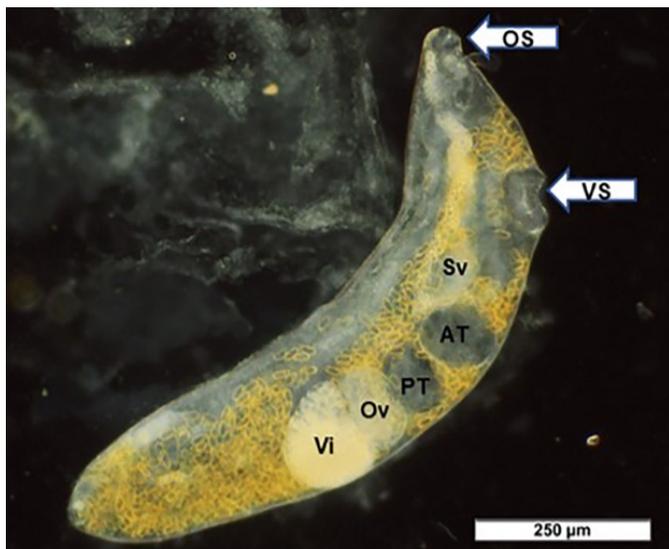


Figure 1. Lateral view of *Aphanurus stossichii* (Monticelli, 1891) in LM

OS: Oral sucker, VS: Ventral sucker, Sv: Seminal vesicle, AT: Anterior testis, PT: Posterior testis, Ov: Ovary, Vi: Vitellarium

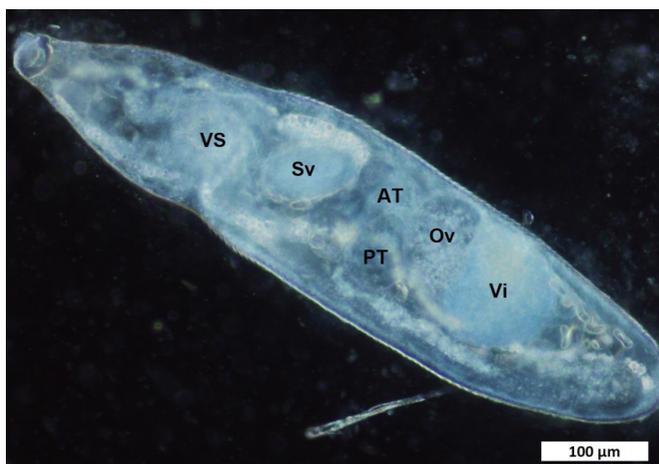


Figure 2. Ventral view of *Aphanurus stossichii* in LM

VS: Ventral sucker, Sv: Seminal vesicle, AT: Anterior testis, PT: Posterior testis, Ov: Ovary, Vi: Vitellarium

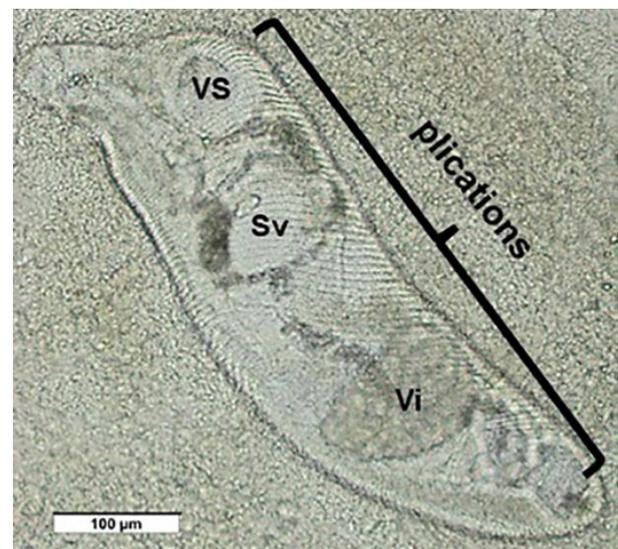


Figure 3. Ventro-lateral view of plications on the specimen in outline

VS: Ventral sucker, Sv: Seminal vesicle, Vi: Vitellarium

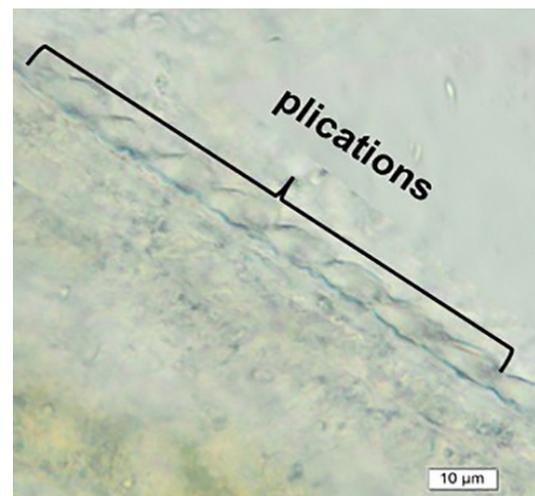


Figure 4. Lateral view of plications in LM

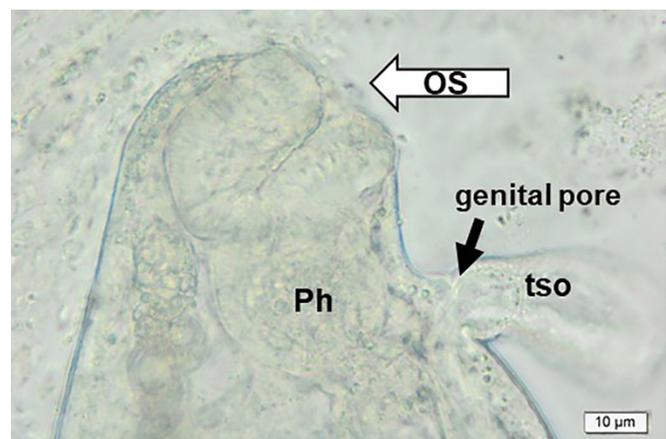


Figure 5. Lateral view of anterior extremity in LM

OS: Oral sucker, Ph: Pharynx, tso: Temporary sinus organ

Scanning Electron Microscopy Observations

Tegument thick, plicated dorsally along the entire length of body but no plication ventrally (Figure 8). In the dorsal part of the body, especially from the level of the ventral sucker to the end of

the hindbody, plications are prominent, but the forebody has no plications either dorsal or ventrally (Figures 8-10). Genital pore median, at mid-level of or immediately posterior to oral sucker (Figure 11). The temporary sinus organ, which is formed from the eversible of the distal half of the hermaphroditic duct, is tubular and covered with small star-shaped tubercles (Figures 12, 13).

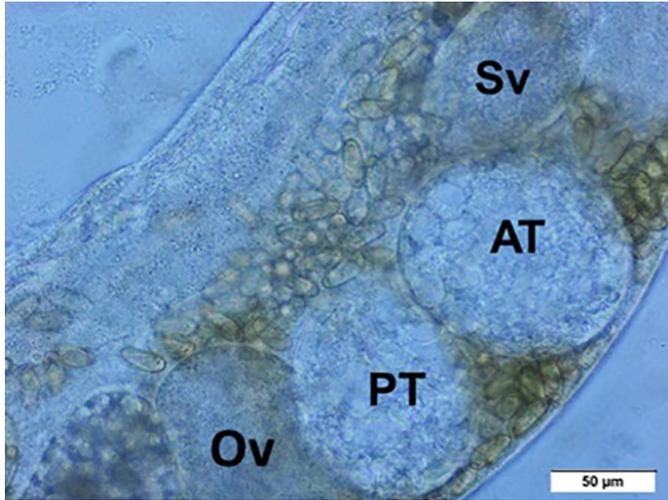


Figure 6. Lateral view of testes in LM
Sv: Seminal vesicle, AT: Anterior testis, PT: Posterior testis, Ov: Ovary

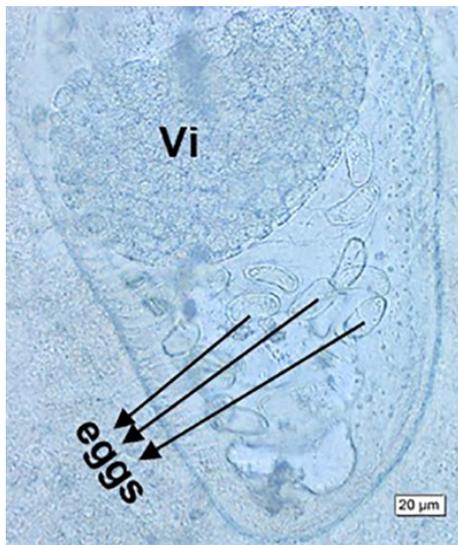


Figure 7. Vitellarium (Vi) and eggs in LM

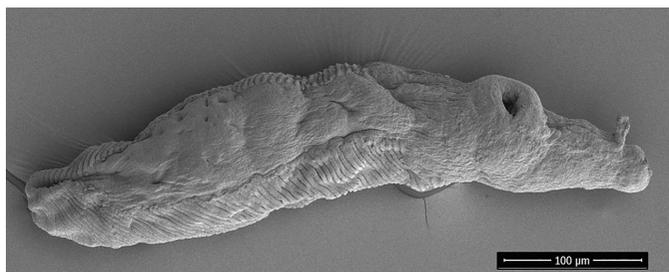


Figure 8. Ventro-lateral view of *Aphanurus stossichii* (Monticelli, 1891) in SEM

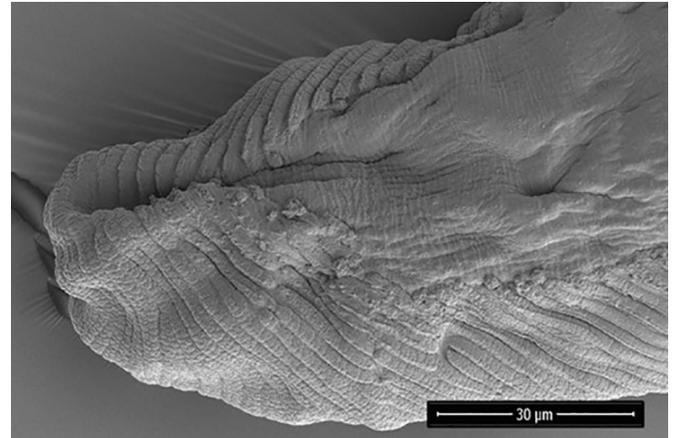


Figure 9. Ventro-lateral view of posterior end in SEM

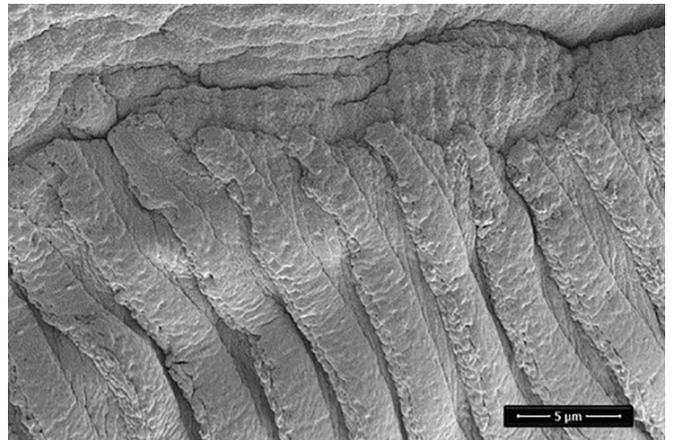


Figure 10. View of tegumental plications in SEM

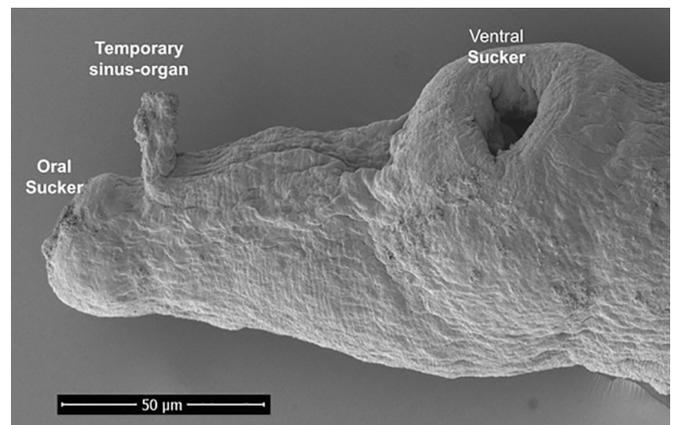


Figure 11. Ventro-lateral view of anterior extremity in SEM

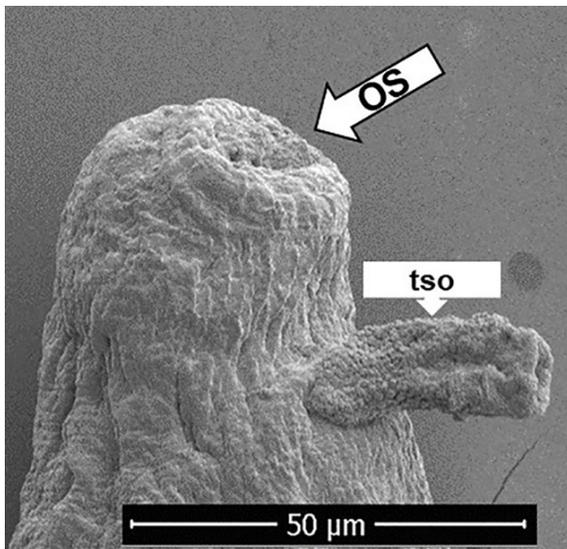


Figure 12. Ventro-lateral view of oral sucker (OS) and temporary sinus organ (tso) in SEM

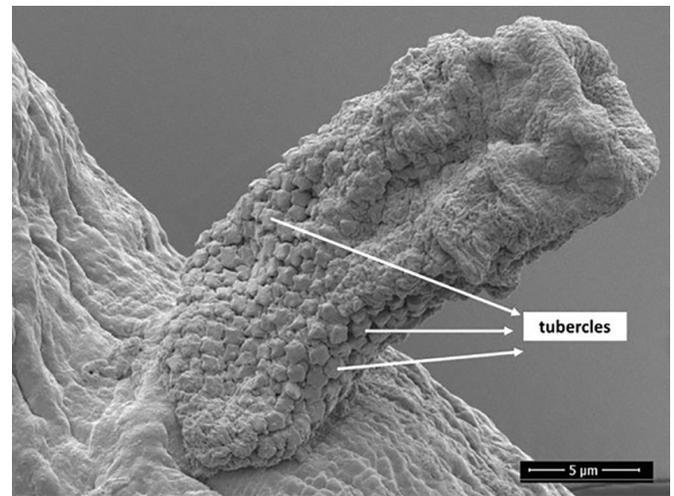


Figure 13. General view of star-shaped tubercles on temporary sinus organ in SEM

Table 1. Comparative morphometric data for *Aphanurus stossichii*

Source	Present study	Dimitrov (6)	Kostadinova et al. (12)			
			<i>E. encrasicolus</i>	<i>B. boops</i>	<i>B. boops</i>	<i>B. boops</i>
Host	<i>E. encrasicolus</i>	<i>E. encrasicolus</i>	<i>E. encrasicolus</i>	<i>B. boops</i>	<i>B. boops</i>	<i>B. boops</i>
Locality	Black Sea (off Sinop, Turkey)	Black Sea (off Bulgaria)	Atlantic (Bay of Biscay)	Black Sea (off Bulgaria)	Mediterranean (off Turkey)	Atlantic (off Spain)
Body length	754-1133 (910)	222-558 (430)	731-917	667-901 (781)	771-1209 (984)	897-1376 (1037)
Body width at ventral sucker	157-226 (201)	44-151 (94)	131-167	163-175 (169)	150-217 (180)	221-300 (248)
Oral sucker length	52-65 (59)	25-47 (34)	40-62	47-55 (51)	51-66 (58)	58-68 (62)
Oral sucker width	55-68 (61)	20-47 (34)	48-62	53-60 (55)	53-72 (60)	58-70 (63)
Pharynx length	35-48 (43)	15-37 (24)	33-51	36-88 (46)	36-49 (44)	40-51 (45)
Pharynx width	31-38 (35)	12-32 (22)	33-58	30-38 (35)	30-47 (38)	36-47 (41)
Ventral sucker length	81-106 (100)	40-91 (63)	81-113	109-132 (122)	104-155 (125)	121-168 (143)
Ventral sucker width	95-122 (110)	35-96 (60)	86-126	128-143 (134)	104-145 (123)	128-160 (145)
Seminal vesicle length	71-110 (97)	20-67 (39)	53-72	62-155 (104)	70-149 (108)	104-145 (124)
Seminal vesicle width	58-88 (76)	10-47 (22)	38-49	68-107 (81)	43-111 (76)	70-115 (90)
Wall of seminal vesicle	6-7	-	3-10	4-13	3-11	2-9
Anterior testis length	65-110 (85)	27-62	66-75	43-72 (57)	47-96 (64)	38-60 (49)
Anterior testis width	76-116 (97)	20-59	73-98	55-113 (83)	51-132 (81)	62-92 (73)
Posterior testis length	60-107 (85)	27-67	55-73	36-70 (57)	43-79 (60)	51-89 (69)
Posterior testis width	70-113 (89)	-	81-92	64-94 (81)	58-121 (87)	70-92 (81)
Ovary length	53-80 (74)	25-49 (37)	49-53	53-79 (65)	51-79 (65)	43-75 (57)
Ovary width	80-140 (129)	20-67 (36)	76-100	70-141 (95)	64-128 (102)	68-124 (86)
Vitellerium length	73-133 (82)	35-74 (49)	81-107	60-92 (81)	53-145 (92)	49-107 (70)
Vitellerium width	98-180 (111)	25-162 (48)	101-136	111-153 (134)	92-181 (128)	89-166 (122)
Egg length	20-26 (23)	-	23-27 (26)	23-28 (25.9)	19-26 (21.9)	26-32 (29)
Egg width	9-11 (10)	-	9-12 (10)	9-13 (11.3)	9-12 (10.5)	9-11 (10)

DISCUSSION

A. stossichii was initially identified as *Distoma ocreatum* by Monticelli (15) from the *Sardina pilchardus* and *Sardinella aurita* (Clupeidae) it was later reported from *Lichia amia*, *Boops boops*, *Spicara manea* and *Trachurus mediterraneus* by Looss (16). This species has recently been redescribed from *Boops boops* from various localities in the North East Atlantic region (off Spain), the Mediterranean Sea (off Spain, and Turkey) and the Black Sea (off Bulgaria). To date, *A. stossichii* has been reported in a variety of fish in the Mediterranean Sea (9,11,12,17-22), in Black Sea (6,12,23), in Atlantic Ocean (10,12,18), in Indian Ocean and Pacific Ocean (24,25). Primarily sparids such as *Boops boops*, *Spicara manea*, and clupeid fish such as *Sardina pilchardus*, *Engraulis encrasicolus*. *A. stossichii* is frequently recorded in the Black Sea and Mediterranean Sea basins and the North East Atlantic. There are differences in morphometric information according to the host and regions that this species is reported. The morphometric data obtained from the samples examined in our study are in agreement with the morphometric data reported by Kostadinova et al. (12) in their study in which *A. stossichii* was redescribed from anchovy in the Bay of Biscay and *Boops boops* in the different basins (see Table 1 for details). However, the morphometric data of *A. stossichii* reported by Dimitrov (6) and the data of this study are not fully coincided, although these data was obtained from the same basin and host (see Table 1).

Aphanurus virgula, which is previously considered a synonym of *A. stossichii*, has been reported in anchovy living in the Black Sea. Because of morphological similarity it is possible that *A. virgula* was reported as *A. stossichii* by Dimitrov (6). Morphometrically, *Aphanurus virgula* is differ from *A. stossichii* by its smaller size and substantially lower range limits for all metrical features, and the eggs being less numerous and larger in relation to the size of the body and gonads. Morphologically, *A. stossichii* can be distinguished from *A. virgula* by having a thicker tegument, more prominent plication, a thicker and larger seminal vesicle, and tubercles in the terminal part of the hermaphrodite duct.

Although there are previously several records about *A. stossichii* in the Black Sea, we can say that this is the first detailed study on the morphological and morphometrical data of *A. stossichii* detected in anchovy in the Black Sea. In previous light microscopy studies; the presence of tubercles on the hermaphroditic duct was noted, but no information was given about the shape of the tubercles. In addition, it has been reported that plications are found in the whole body of the parasite individual by light microscopy. Similarly, in our light microscopy examinations, we thought all over the body of the parasite had plications. Unlike the appearance of plications under the light microscope, our SEM examinations revealed that the plications did not cover the entire body of the parasite, it interrupted in the abdomen of the parasite. This new finding obtained by SEM is important in terms of contributing to the definition of the morphological features of this species.

CONCLUSION

In the present study, we provided the first SEM data on morphological observations of *A. stossichii* infecting the European anchovy, *Engraulis encrasicolus* off the Turkish coasts of the Black Sea. All the illustrations and morphometric data presented here

make further contributions to our current knowledge and also provide a base for further studies.

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Some parts of this study were previously presented as posters in an international symposium (International Congress on Engineering and Life Science/26-29 April 2018/Kastamonu/Turkey).

* Ethics

Ethics Committee Approval: During the study, no treatment/experiment was implemented on the live animal. All sampling and laboratory work on fish have complied with the Republic of Turkey Ministry of Agriculture and Forestry animal welfare laws.

Informed Consent: During the study, no treatment/experiment was implemented on the live animal. All sampling and laboratory work on fish have complied with the Republic of Turkey Ministry of Agriculture and Forestry animal welfare laws.

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* Authorship Contributions

Surgical and Medical Practices: T.Ö., A.G., Concept: T.Ö., A.G., Design: T.Ö., A.G., Data Collection or Processing: T.Ö., A.G., Analysis or Interpretation: T.Ö., A.G., Literature Search: T.Ö., A.G., Writing: T.Ö., A.G.

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