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RESEARCH ARTICLE

Camachoaglaja africana Pruvot-Fol, 1953 (Gastropoda, Heterobranchia, Cephalaspidea) in Libya: first records and notes on its mating behaviour

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Abstract

Camachoaglaja africana is a small sea slug found in Macaronesia, Atlantic coasts of North Africa and the Mediterranean Sea. This mollusc can be found in shallow waters up to 30 meters deep. During field surveys conducted to assess sea slug biodiversity in Libyan waters, *C. africana* was discovered in three different locations along the western coast of Libya. The mating season and behaviour of this species were also documented in this study. The present record expands the known distribution of *C. africana* to include Libya and the African Mediterranean coast.

Keywords: Spawning, hermaphrodite, North Africa, Heterobranchia

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Introduction

The Aglajidae *Camachoaglaja africana* (Pruvot-Fol, 1953) (synonym *Chelidonura africana* Pruvot-Fol, 1953; *Chelidonura italica* Sordi, 1980; *Chelidonura leopoldoi* Ortea, Moro & Espinosa, 1997) is a small sea slug that can be easily overlooked by the untrained eye. The elongate and straight sea slug measures between 1-15 mm (Perrone and Sammut 1997; Sordi 1980). The animal ranges in colour from brownish-black to black, with some lighter specimens described as dirty white (Trainito *et al.* 2023). In darker specimens, tiny scattered white spots cover the body, which tend to accumulate on the back end of the cephalic shield forming a wide white band (Sordi 1980; Perrone and Sammut 1997). This can be absent in some specimens (Sordi 1980; Rudman 2003). Turquoise spots can be seen on the side walls of the parapodia and caudal processes. The headshield takes up almost half of the entire length. The foot,

convex along its entire length, is not distinct from the rather small parapodia, covering the sides and, to a limited extent, the back (Sordi 1980). Two groups of sensory cilia are clearly seen at the bilobed anterior edge of the head. The cephalic shield is bilobed anteriorly and rounded posteriorly. For further detailed description, see Sordi (1980) and Perrone and Sammut (1997).

Camachoaglaja africana is known to prefer areas abundant with algae and invertebrates (Ballesteros *et al.* 2020) on which it preys. Additionally, it has been reported from soft bottoms (Furfaro *et al.* 2020) and under rocks (Martinez *et al.* 2002). Sordi (1980) and Perrone and Sammut (1997) reported it in shallow waters. However, it has been known to inhabit depths of up to 30 meters (Furfaro *et al.* 2020).

Similar to other molluscs, *C. africana* is a simultaneous hermaphrodite (Garcia and Garcia 1984) with an ovotestis (Sordi 1980). Garcia and Garcia (1984) observed the mating behaviour of two *C. africana* under laboratory conditions. Mating occurs between two individuals who position themselves parallel to one another, with the sperm donor slightly behind the sperm receiver. The sperm donor inserts its head under the parapodia. They then switch roles, with the sperm donor becoming the sperm receiver and vice versa.

Camachoaglaja africana has been documented in various locations. These include the Atlantic coast of Morocco (Pruvot-Fol 1953; Martinez *et al*. 2002), Senegal (Pruvot-Fol 1953), Madeira (Martinez *et al*. 2002) the Azores (Malaquias *et al*. 2009), the Canary Islands (Ortea *et al.* 1996; Martinez *et al*. 2002), and Portugal (Cervera *et al*. 2004). In the Mediterranean Sea *C. africana* has been observed in the Strait of Gibraltar (Garcia and Garcia 1984; Rudman 2003), along the Spanish coastline (Ballesteros *et al.* 1986), Malta (Perrone and Sammut 1997), Croatia (Rudman 2006; Turk and Furlan 2011), France (Ballesteros *et al.* 2020), Greece (Manousis *et al*. 2020) and Bosnia and Herzegovina (Kahrić *et al*. 2023). In Italy, *C. africana* has been documented in various locations since it was first recorded in Secche della Meloria, Livorno (Sordi 1980). These include Cagliari, Sardinia (Rudman 2005), Sicilia (Villari and Scuderi 2017; Lombardo and Marletta 2020), Porto Pirrone (Furfaro *et al*. 2020), Campomarino (Furfaro *et al*. 2020), Isola dei Conigli, Lampedusa (Furfaro *et al*. 2020) and the marine protected area Tavolara Punta Coda Cavallo (Trainito *et al.* 2023). There have been no records of this species in Libya, or its neighboring North African countries facing the Mediterranean Sea.

This could be attributed to the scarcity of malacological studies in Libya with little information on Gastropoda (Rizgalla *et al.* 2018). In response, a project called Project Snowball was initiated to assess marine biodiversity in Libyan waters (Rizgalla and Crocetta 2020). As part of this initiative, a seven-year ongoing study was launched to assess sea slug biodiversity in Libyan waters resulting in the addition of several new species to the list of Gastropoda in Libya

(e.g. Rizgalla *et al*. 2019a, b, 2023; Rizgalla and Crocetta 2020; Rizgalla 2025). The present study provides a first confirmed record of *C. africana* in three locations in the west of Libya, with notes on its mating behaviour, marking the first record of this species in Libyan waters.

Materials and Methods

Surveys included three locations along the Libyan coastline between January and October, 2018-2025 (see Table 1). These were undertaken during the day, and typically lasted for 2-4 hours. In Tripoli, two locations were surveyed. The first was the natural bay in Abonawas, which has a mixed sandy and rocky substrate with several tidal pools forming during low tides. *Caulerpa racemosa* (Forsskål) J. Agardh, seemed to be the predominant alga growing in the bay with a maximum depth of 50 to 70 cm (Figures 1A, B and C; 32°51'57.6"N 13°05'30.1"E). Substrate was collected from tidal pools and from the bay. The second survey in Tripoli was conducted at Regatta (Figures 1A and D; 32°51'13.7"N 13°03'13.9"E). Regatta is a natural bay with sandy and rocky substrate. It was surveyed by snorkelling, direct collection of specimens, and substrate collections. Substrates included the bryozoan *Amathia verticillata* (delle Chiaje, 1822), various algae, including *Halopteris scoparia* (Linnaeus) Sauvageau. The third survey area is called Surman (Figures 1A and E; 32°47'47.7"N 12°33'54.3"E), a mixed sandy to rocky natural bay. Snorkelling, in addition to collecting various substrate including *Laurencia obtusa* (Hudson) J. V. Lamouroux and *Jania rubens* (Linnaeus) J.V. Lamouroux.

Table 1. Survey months and locations of three survey locations in 2018-2025.

Year	Survey locations		
	Abonawas	Regatta	Surman
2018	-	June to Nov.	-
2019	-	Apr. to Oct.	-
2020	-	-	-
2021	-	May to Sept.	Sept.
2022	-	May to Sept.	-
2023	-	Apr. to Oct.	Sept. & Oct.
2024	May& June	Mar. to July	Mar. to Aug.
2025	Jan.	-	Jan.

The substrates were kept in a room with dim light in non-aerated plastic containers filled with sea water for 48 hours at room temperature and were periodically screened with the help of a strong light source. The specimens were found on the sides or bottom of the plastic containers, as well as among the algae (Table 2). They were then transferred to non-aerated plastic containers with sea water, and kept for up to five days at ambient temperature. Pictures and videos of the live specimens were taken using an Olympus Tough TG-4 Underwater Camera. Specimens were observed under a dissecting (Hamillton) and compound microscope (Carl Zeiss Axiostar) and photographed, measured alive to the nearest

1 mm, and then fixed in 96% ethanol. The survey was interrupted in 2020 due to the global pandemic and to local armed conflict.

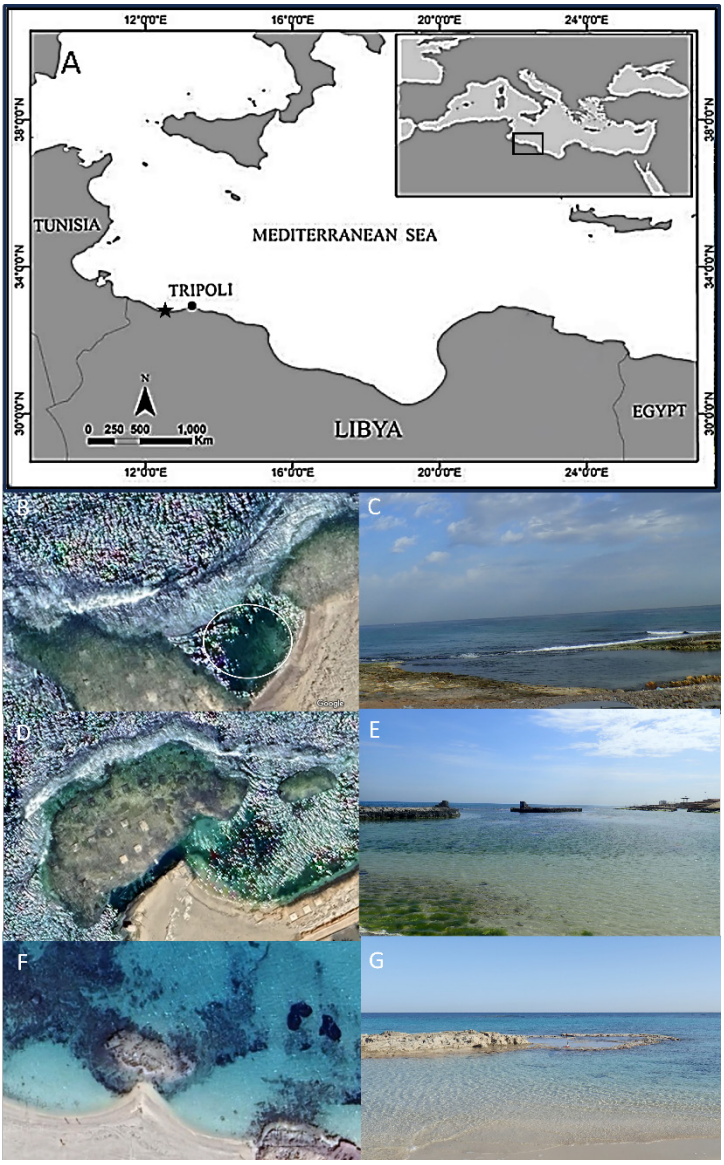

Figure 1. (A) The map of Libya highlights surveyed locations in Tripoli (black dot) and Surman (black star); (B) a satellite image of Abonawas (google map); (C) a natural bay Abonawas, indicating survey area (white circle in B); (D) a satellite image of Regatta (google map); (E) a natural bay Regatta; (F) a satellite image of Surman survey area (google map); (G) tidal pools in a natural bay Surman during low tide.

Results

A total of twenty *C. africana* were collected between March and September from the three locations as shown in Figures 1, 2 and Table 1. The specimens included juveniles and adults ranging in sizes between 0.8-5.1 mm (Table 2). *Camachoaglaja africana* were found beneath rocks, among algae, or among the alive and decaying bryozoan *A. verticillata* at depths ranging between 10 -70 cm. A spherical egg mass was found under a rock close to where *C. africana* was spotted (Figure 2A). On July 27, 2022 a specimen of *C. africana*, which was sampled on July 25, 2022, laid a spherical egg mass at the bottom of a plastic container filled with sea water (Figure 2B).

Table 2. List of twenty specimens of *Camachoaglaja africana* with sampling details, found in Abonawas (A), Regatta (R) and Surman (S)

Location	Figure	Date	Size (mm)	Depth (cm)	Substrate*
R	2K	29 Mar 24	1.3	50	UA
S		05 Apr 24	1.5	30-50	UA
R	2F	06 May 23	3	30-50	UA
A	2C	13 May 24	5.1	30-50	*C. racemosa*
A	2D	13 May 24	3	10-30	*C. racemosa*
A	2E	19 Jun 24	4.5	10-30	*C. racemosa*
R		14 Jul 24	3.5	50	UA
R		21 Jul 23	3.5	30-50	*A. verticillata*
R	2G	22 Jul 23	4	30-50	*A. verticillata*
R		23 Jul 22	3	50	Under a rock
R	2H	25 Jul 22	3	50	*A. verticillata*
S		29 July 24	2	50	*J. rubens* & UA
S	3A (i)	01 Aug 24	2.5	30-50	*J. ruben* & UAs
S	3B (iii)	01 Aug 24	3.5	30-50	UA
S	3A (ii)	01 Aug 24	3	30-50	*Laurencia obtuse, J. rubens* & UA
R		02 Aug 22	2.9	40	UA
R	2I	02 Aug 22	2	50-70	UA
R	2J	02 Aug 22	1.8	40	UA
S		12 Aug 24	2.5	40-50	UA
R	2L	16 Sep 23	0.8	30-50	UA

*Abbreviation: UA unidentified alga

The morphology and variation in colour patterns of the specimens agrees with descriptions provided by Pruvot-Fol (1953), Perrone and Sammut (1997), Rudman (2003) and Martinez *et al.* (2002). These include: i. The dark black (Figure 2A) or brown (Figures 2E and F) homogenous background colour sprinkled white spots of varying diameter, ranging from minute (Figures 2B and E) to larger spots (Figure 2D). Some specimens appear lighter than others (Trainito *et al.* 2023) with the background colour fading to almost a dirty white (Figure 2H and I). ii. Two large parapodia, tapering at the end (tails) with one longer than the other, were consistent among most specimens found. They can

also be very long on one side (Figures 2F and 3C), or equally long (which could be due to injury) (Figure 2G). The cephalic shield is roughly triangular. iii. A transversal yellow line on the head behind the white band was present in larger specimens but less visible in younger ones. A yellow thin line demarcates the border of the parapodial lobes and the caudal lobe. iv. Vivid electric blue (Figures 2C and D) to light blue or almost turquoise (Figure 2E) roughly quadrangular spots on the edge of the external margins of the parapodia.

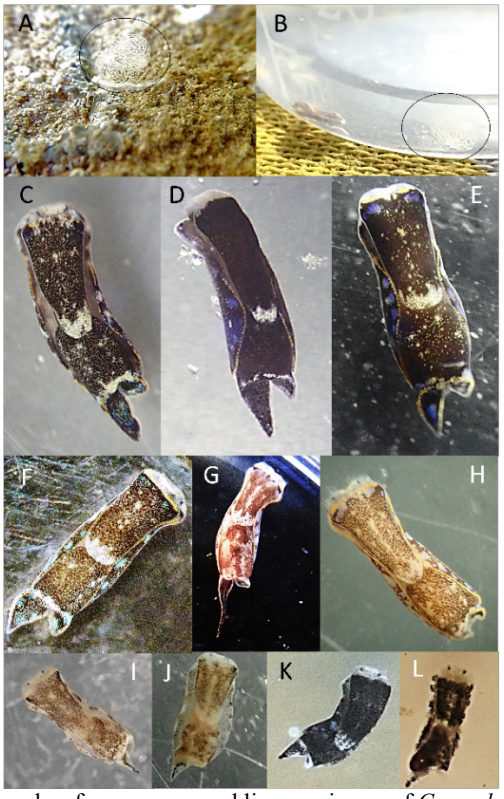

Figure 2. Photographs of egg masses and live specimens of *Camachoaglaja africana.* (A) Spherical egg mass found under a rock at 30 cm depth (black circle); (B) a loose spherical egg mass lied at the side of a plastic container (black circle) approximately 3 mm wide, alongside it at the bottom of the container the sea slug found on the July 25, 2022 (specimen H); (C) a 5 mm specimen, with a dark background sprinkled with minute white dots; (D) a dark almost black specimen 4 mm, with electric blue dots on the edge of the external margins of the parapodia; (E) a dark specimen 4.5 mm, with electric blue dots; (F) a 3 mm specimen with many tiny white dots and light blue (turquoise) dots on the edge of the external margins of the parapodia; (G) a 4 mm specimen with one long parapodia; (H) a 3 mm specimen with two equally long parapodia almost rounded at the end (possibly caused by injury); (I) a small specimen (2 mm) with dirty white background and minute black dots; (J) a second pale specimen (1.8 mm); (K) a specimen with dark background colour (1.3 mm); (L) A small individual (0.8 mm).

Mating

Three specimens of *C. africana* sampled on August 1, 2024, referred to as: i, ii and iii, were transferred into a Petri dish on August 2, 2022. Shortly after, two of them (i and ii) initiated courtship, positioning themselves parallel to each another (Figure 3A). Thereafter, all three formed a spawning aggregation, forming a spawning chain, where all three appearing to engage in mating (Figure 3C). The third specimen (iii) separated after less than a minute. The now mating pair, i and ii, continued mating (Figure 3D). The sperm donor (ii) positioned its head at roughly 45 degrees between the posterior shield and the right parapodium of the sperm recipient (i) while the copulatory organ remained inserted under the mantle (Figure 3E) similar to the description provided for *C. africana* (*Chelidonura africana*) (Garcia and Garcia 1984) and for C*helidonura sandrana* Rudman, 1973 (Anthes *et al*. 2004; Anthes and Michiels 2007). The mating partners alternated their sex role as either, sperm donor or recipient (male or female role), rather than mating simultaneously. The second mating cycle between the now sperm donor (i) and sperm recipient (ii), lasted 12 minutes, from 19:34-19:46 (Figure 3E), at which point the mating pair separated by moving away from one another. The penis appeared translucent white (Figure 3E).

Discussion

Camachoaglaja africana were present from March to September, as both juvenile and adult specimens. Ballesteros *et al.* (2020) reported the presence of *C. africana* all year, except for November. This discrepancy in the present study could be attributed to its small size and the lack of surveys in November and January. Older individuals were observed from March to September, while younger individuals were seen between July and August. The size range of *C. africana* reported herein, 0.8 mm to 5 mm, is in agreement with similar observations (Ballesteros *et al*. 2020). However larger individuals reported from this species were not found during the surveys.

The morphology of the specimens described in this study agrees with descriptions provided by Pruvot-Fol (1953), Perrone and Sammut (1997), Martinez *et al*. (2002) and Rudman (2003). Furthermore, the chromatic variation in *C. africana* background colour intensity observed in twenty *C. africana* specimens ranged from dark to almost dirty white, a colour variation frequently reported in the literature (Garcia and Garcia 1984; Rudman 2003; Trainito *et al*. 2023).

Camachoaglaja africana was often found in association with decaying substrates, especially *A. verticillata,* widely present in Regatta between July and August (Rizgalla *et al.* 2019b) but not present in other two survey locations. *Camachoaglaja africana* were also found among *L. obtusa* and *J. rubens*, in accordance with Perrone and Sammut (1997), both of which were widely distributed in Surman (El Nass *et al*. 2024), but also among *C. racemosa,* widely present in Abonawas.

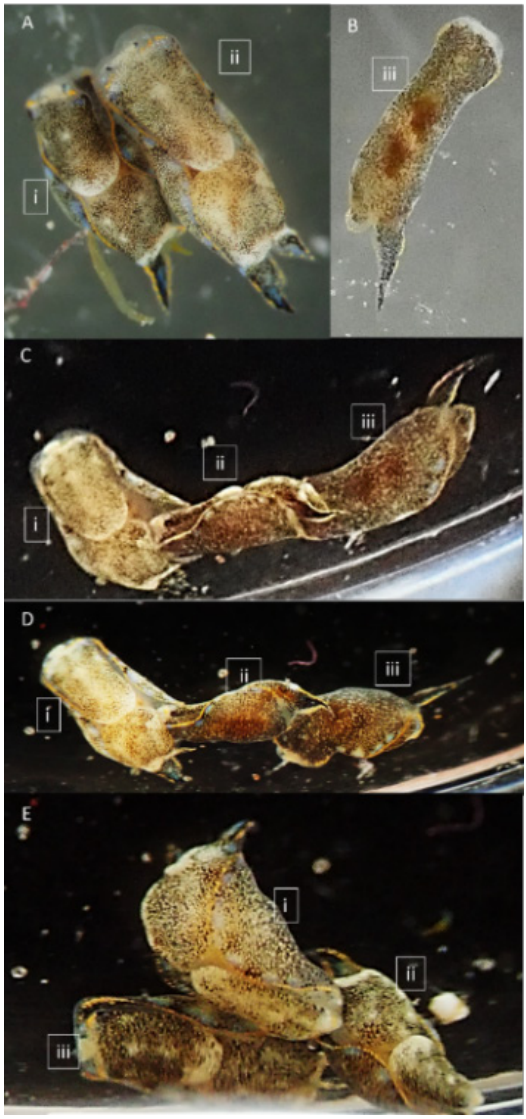

Figure 3. Mating behaviour of *Camachoaglaja africana* observed on August 1, 2024. (A) Two specimens (dorsal view), side by side in preparation for mating (i: 2.5 mm; ii: 3 mm); (B) abdominal view (iii: 3.5 mm); (C) three specimens (i, ii, iii) forming a mating chain, mating constellation: terminal sperm recipient (i), sperm donor and sperm recipient (ii), terminal sperm donor (iii); (D) terminal sperm donor (iii) stops mating, remaining close to the mating pair (i&ii); (E) sex role alternation, reversed mating position, with sperm recipient, assuming the sperm donor role (ii) seen inserting its copulatory organ under the mantel of the sperm receiver (i) formally sperm donor.

Mating strategies vary greatly among sea slug species (Angeloni and Bradbury 1999; Anthes and Michiels 2007). In some Aglajidae species, when three individuals are present, they form a mating chain by trailing after one another: terminal sperm recipient, sperm donor and recipient, and terminal sperm donor, as observed in *C. africana* in the present study. Furthermore, *C. africana* mating also involved only two individuals (Garcia and Garcia 1984). Both larger and smaller *C. africana* individuals alternate sex roles, from sperm donor to recipient and vice versa, similar to observations made by Garcia and Garcia (1984) and in other Aglajidae species such as *C. sandrana* (Anthes and Michiels 2007). However, in contrast to some Aglajidae species (Anthes and Michiels 2007), reciprocal insemination was not observed during *C. africana* mating.

Egg masses were observed in the wild and laid under laboratory conditions in late July. The egg masses in accordance with other Aglajidae species were spherical in shape (Anthes and Michiels 2007).

This is the first documented record of *C. africana* in Libyan waters. Due to its abundance in all three surveyed locations, it suggests a more common presence within its geographical range. Further research is necessary to evaluate its distribution along the western, central and eastern Libyan coast.

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