

Sperm ultrastructure in the family Opecoelidae (Digenea): first data on the genus *Opegaster*

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INTRODUCTION & AIM

Ultrastructural studies of spermatozoa have proven to be a valuable source of characters for resolving phylogenetic relationships within parasitic Platyhelminthes. In digeneans, sperm ultrastructure has been extensively used to support systematic and evolutionary hypotheses, particularly through comparative spermatology. The organization and the number of characters such as axonemes, cortical microtubules, mitochondria, and external ornamentation of the plasma membrane have shown strong phylogenetic signal.

Within the family Opecoelidae, ultrastructural spermatological data remain limited. To date, only thirteen species representing five subfamilies have been investigated, leaving large gaps in our understanding of sperm diversity within the group. The subfamily Opecoelinae, although taxonomically diverse, is still poorly represented in ultrastructural studies, and no data were previously available for the genus *Opegaster*.



Fig. 1: Giant mudskipper [1].

The present study addresses this gap by providing the first detailed description of spermatozoon ultrastructure in *Opegaster* sp., a digenean parasite collected from the giant mudskipper *Periophthalmodon schlosseri* (Fig. 1) in Thailand. The objectives of this study were to describe the sperm ultrastructure of *Opegaster* sp. using TEM and compare its spermatological characters with those of other Opecoelidae, particularly Opecoelinae.

METHOD

Specimens: Digeneans were collected from the intestine of a brackish-water, giant mudskipper, *Periophthalmodon schlosseri* (Pallas, 1770) (Gobiformes: Oxudercidae), captured in April 2025 in a Mangrove area (Fig. 2), Chumphon Province, Thailand. Fish collecting was conducted in accordance with the local legislation and institutional requirements of the Animal Care and Use Committee of King Mongkut's Institute of Technology Ladkrabang, Thailand (Approval No. ACUC-KMITL RES/2025/009).



Fig. 2: Mangrove area where the giant mudskipper was collected.

Transmission Electron Microscopy: Live worms were fixed in 2.5% glutaraldehyde in a 0.1 M sodium cacodylate buffer (pH 7.4), rinsed in the same buffer, postfixed in 1% osmium tetroxide in the buffer, rinsed in MilliQ water, dehydrated in an ethanol series and propylene oxide, embedded in Spurr's resin, and finally polymerized at 60°C. Ultrathin sections mounted on copper and gold grids were double-stained with uranyl acetate and lead citrate and examined using a JEOL 1010 transmission electron microscope at the "Centres Científics i Tecnològics" of the University of Barcelona (CCiTUB).

RESULTS & DISCUSSION

The spermatozoa of *Opegaster* sp. are filiform and tapering at both extremities. They exhibit the ultrastructural features typically observed in most digeneans. Two axonemes of unequal length are present and display the trepaxonematan 9+1' pattern. Parallel cortical microtubules are arranged beneath the plasma membrane. Additional structures observed include external ornamentation of the plasma membrane, spine-like bodies, mitochondria, and a nucleus. Lateral expansions were not observed at any level of the spermatozoon (Fig. 3).

The distribution and organization of these characters along the sperm cell are generally consistent with those reported for other opecoelid species. Based on the combination of ultrastructural features, the spermatozoon of *Opegaster* sp. corresponds to the type III sperm pattern as defined by Bakhoum et al. (2017) [2].

The sperm ultrastructure of *Opegaster* sp. shows strong conformity with the general spermatological pattern described for the family Opecoelidae (Table 1). The presence of two trepaxonematan axonemes, parallel cortical microtubules, mitochondria, spine-like bodies, and external ornamentation supports its placement within the Digenea and confirms the conservative nature of several sperm characters at the family level.

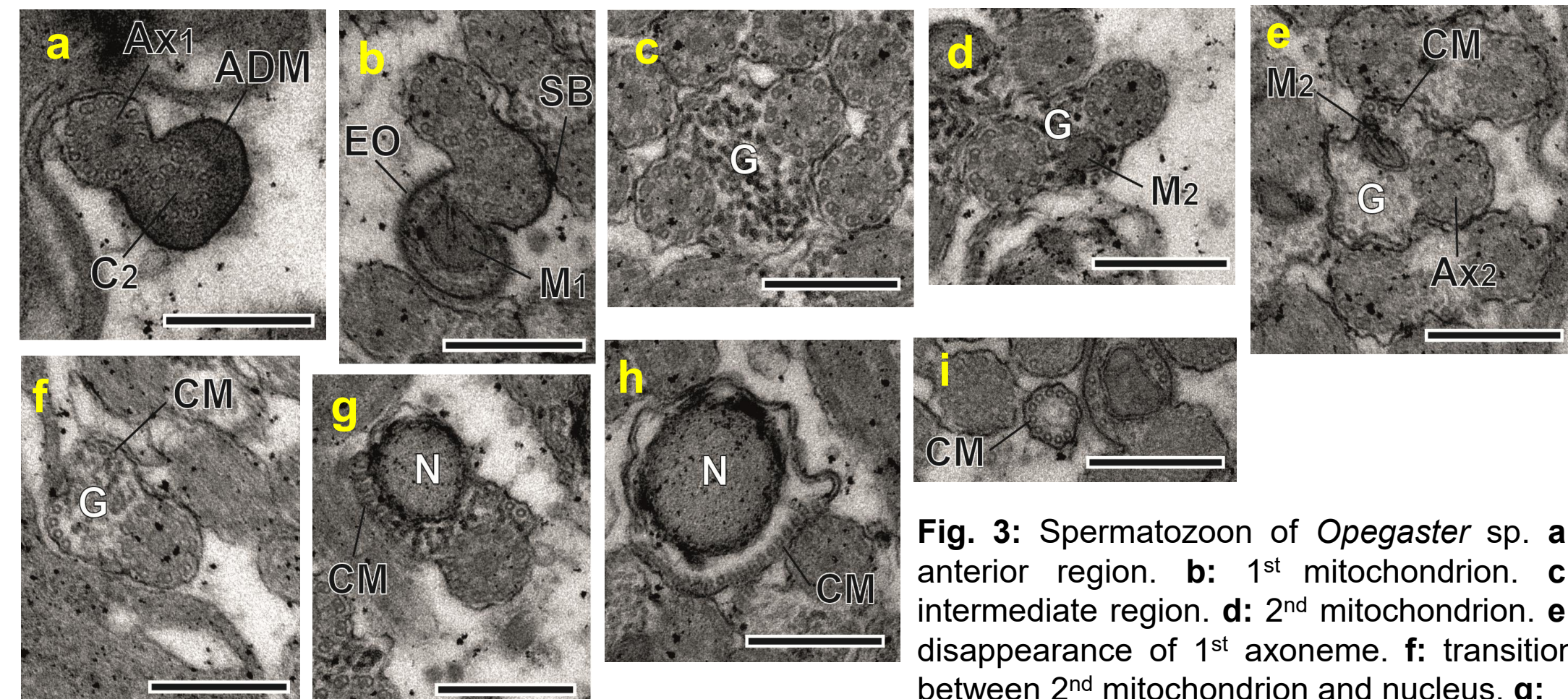


Fig. 3: Spermatozoon of *Opegaster* sp. a: anterior region. b: 1st mitochondrion. c: intermediate region. d: 2nd mitochondrion. e: disappearance of 1st axoneme. f: transition between 2nd mitochondrion and nucleus. g: nuclear region. h: disappearance of 2nd axoneme. i: cortical microtubules in posterior sperm extremity. Scales: 300 nm. ADM: anterior electron-dense material; Ax1, Ax2: axonemes; CM: cortical microtubules; EO: external ornamentation of the plasma membrane; G: glycogen; M1, M2: mitochondria; N: nucleus; SB: spine-like bodies.

nuclear region. h: disappearance of 2nd axoneme. i: cortical microtubules in posterior sperm extremity. Scales: 300 nm. ADM: anterior electron-dense material; Ax1, Ax2: axonemes; CM: cortical microtubules; EO: external ornamentation of the plasma membrane; G: glycogen; M1, M2: mitochondria; N: nucleus; SB: spine-like bodies.

Table 1: Sperm characteristics in the Opecoelidae. Data from previous studies [3]. ADM: anterior electron-dense material; AntS: anterior region of the spermatozoon; CM: cortical microtubules; EO: external ornamentation of the plasma membrane; EO+CM: external ornamentation–cortical microtubules association; LE: lateral expansion; LEO: location of external ornamentation; LMCM: location of maximum number of cortical microtubules; M: number of mitochondria; MedS: median region of the spermatozoon; N: nucleus; NA: not applicable; PostA: posterior part of anterior region; PostS: posterior region of the spermatozoon; PSC: posterior spermatozoon character; SB: spine-like bodies; +: presence of considered character; -: absence of considered character.

	Sperm characters								
	LE	EO	EO+CM	LEO	LMCM	M	ADM	SB	PSC
Hamacreadiinae									
• <i>Allopodocotyle pedicellata</i>	–	+	+	PostA	AntS	2	+	+	CM
• <i>Allopodocotyle tunisiensis</i>	–	+	+	PostA	AntS	2	+	+	CM
• <i>Podocotylodes magnatestis</i>	–	+	+	PostA	MedS	2	+	+	N
Helicometrinae									
• <i>Helicometra epinepheli</i>	–	+	+	PostA	MedS	2	+	+	CM
• <i>Helicometra fasciata</i>	–	–	NA	NA	MedS	2	+	–	CM
Opecoelinae									
• <i>Labracetabulum gephyroberici</i>	–	+	+	PostA	MedS	2	+	+	CM
• <i>Opecoeloides furcatus</i>	–	+	+	PostA	MedS	2	–	+	CM
• <i>Opegaster</i> sp.	–	+	+	PostA	MedS	2	+	+	CM
• <i>Poracanthium furcatus</i>	–	+	+	PostA	MedS	2	+	+	CM
Opistholebetinae									
• <i>Heterolebes maculosus</i>	–	+	+	PostA	MedS	2	–	+	CM
• <i>Macvicaria obovata</i>	–	+	+	PostA	PostS	2	+	+	CM
• <i>Peracreadium characis</i>	–	–	NA	NA	AntS	2	+	–	CM
Plagioporinae									
• <i>Nicolla testiobliqua</i>	–	+	+	PostA	MedS	2	–	+	CM
• <i>Nicolla wisniewskii</i>	–	+	+	PostA	MedS	2	–	+	CM

Within the subfamily Opecoelinae, the identification of a type III sperm pattern in *Opegaster* sp. is consistent with observations in *Labracetabulum gephyroberici*, *Opecoeloides furcatus*, and *Poracanthium furcatus*. This suggests a relative homogeneity of sperm ultrastructure within the subfamily.

However, comparisons across opecoelid subfamilies reveal some discrepancies in the presence, absence, or distribution of specific characters, indicating that spermatological traits may still provide useful resolution at lower taxonomic levels. The new data for *Opegaster* sp. contribute to refining comparative frameworks used in digenean phylogenetics.

CONCLUSION

This study provides the first ultrastructural description of the spermatozoon in the genus *Opegaster*. The observed spermatological characters are consistent with those of other members of the Opecoelidae and support the assignment of *Opegaster* sp. to the type III sperm pattern within the Opecoelinae.

These findings expand the existing dataset on opecoelid sperm ultrastructure and reinforce the value of spermatological characters in systematic and phylogenetic studies of digeneans. Further ultrastructural investigations across additional taxa are needed to better understand character evolution within the family.

FUTURE WORK / REFERENCES

- [1] https://wildkratts.fandom.com/wiki/Giant_Mudskipper
[2] Bakhoum et al. Adv. Parasitol. 2017, 98, 111-165.
[3] Kacem and Miquel. Animals. 2023, 13, 1953.