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Exploring the secretome of *Schistocephalus solidus*: Extracellular vesicles for host manipulation

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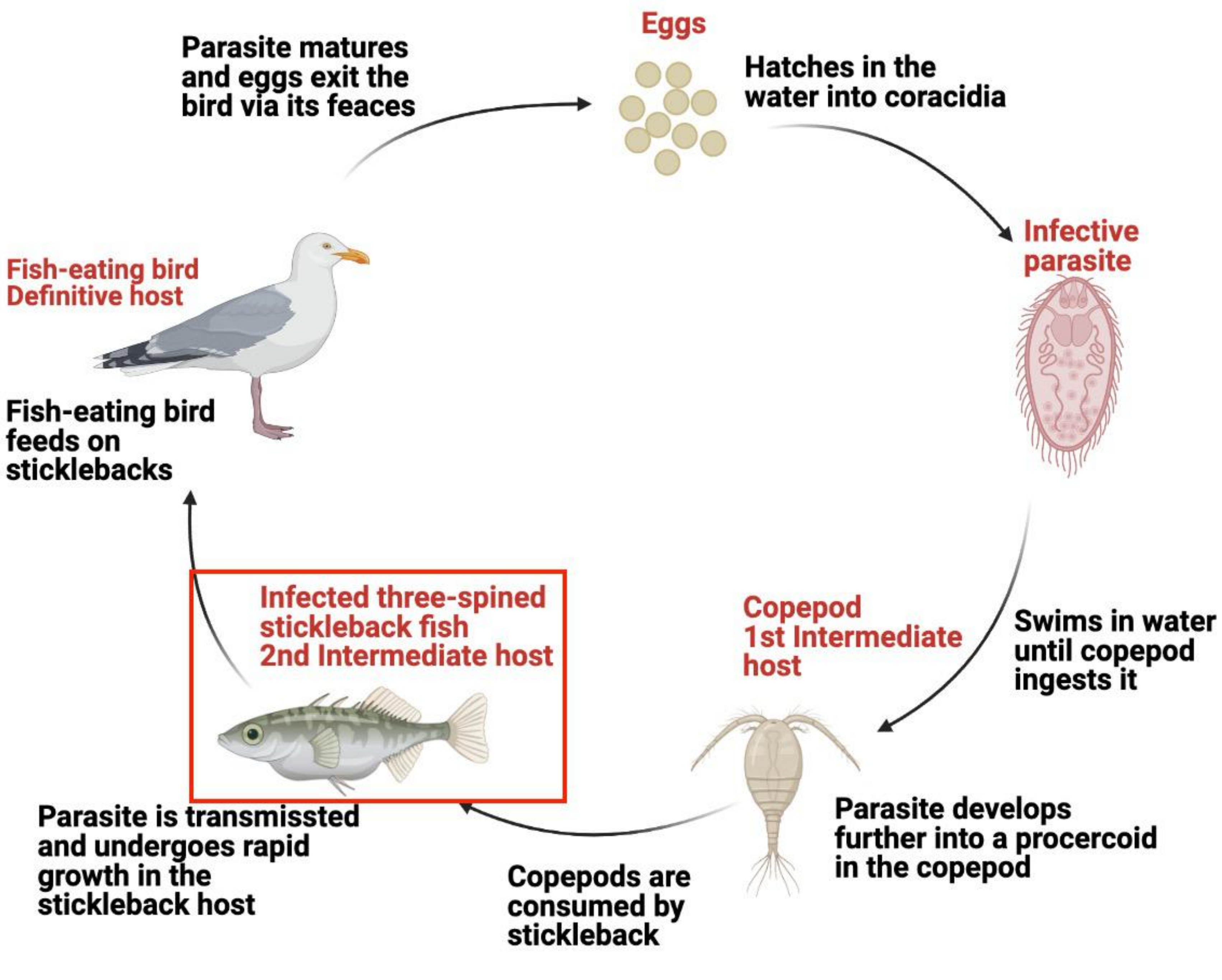


Figure 1- Lifecycle of Schistocephalus solidus, behavioural manipulation caused by the cestode can be observed in both intermediate hosts, the copepod and stickleback fish. This project is focused on the 2nd intermediate host (boxed).

Background

To promote parasite transmission, the cestode S. solidus has been observed to manipulate the behaviour of their secondary intermediate host (*Figure 1*), the three-spined stickleback (*Gasterosteus aculeatus*). In doing so, the host undertakes more risk-taking behaviour such as:

- **positive geotaxis**[1] (gravitates towards stimulation)
- **negative thigmotaxis**[2] (physical discontinuity to environment)

The change of behaviour occurs at the molecular level, rather than from the physical presence of the parasite within the body cavity of the host[3] (*Figure 2*). Excretory secretory proteins (ESP) produced at the plerocercoid stage have been examined in relative depth and has yielded novel putative host manipulating proteins. However, a crucial part of the secretome has been neglected, namely the presence, or absence, of extracellular vesicles (EVs). **EVs have been widely found to be secreted from many helminths including both trematode and cestode flatworms[4].**

Figure 2- Schistocephalus solidus found in the abdomen of its stickleback host.[5]

Aim

Given that the molecular mechanism of how *S. solidus* is unknown, it is likely that *S. solidus* derived EVs act as a crucial host manipulator. **This project aims to expand the known secretome of *S. solidus* and explore both EVs and ESP, depleted of EVs, that may act as putative host manipulators.**

Methods

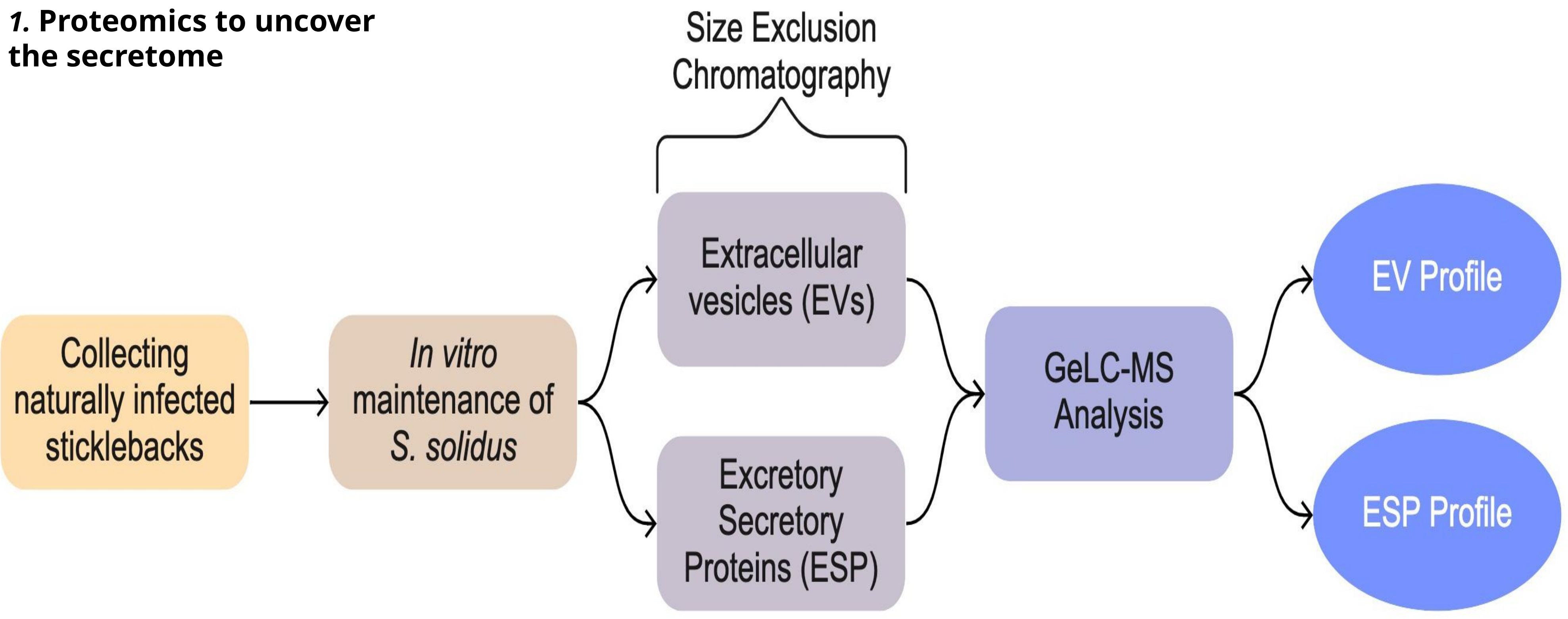
1. Proteomics to uncover the secretome

- *S. solidus* parasites were collected from naturally infected sticklebacks
- *In vitro* maintenance of *S. solidus* at 4℃ and 39℃ to emulate the living conditions within the stickleback and fish-eating bird respectively
- EVs and ESP were purified using Size Exclusion Chromatography (SEC)
- Both samples were then subjected to GeLC-MS analysis
- EVs and ESP profiles were then generated from the results

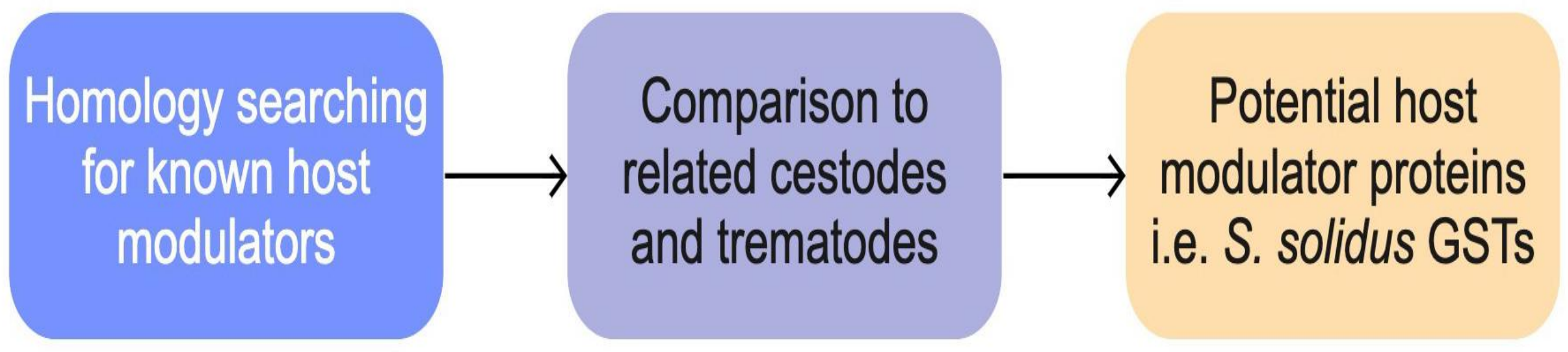
2. Bioinformatics to identify host modulators

- Homology searching of the *S. solidus* genome using known host modulators, such as the Glutathione transferases (GSTs)
- Comparison of GST superfamily present in *S. solidus* to previous searched cestode and trematode secretomes

1. Proteomics to uncover the secretome



2. Bioinformatics to identify host modulators



Results

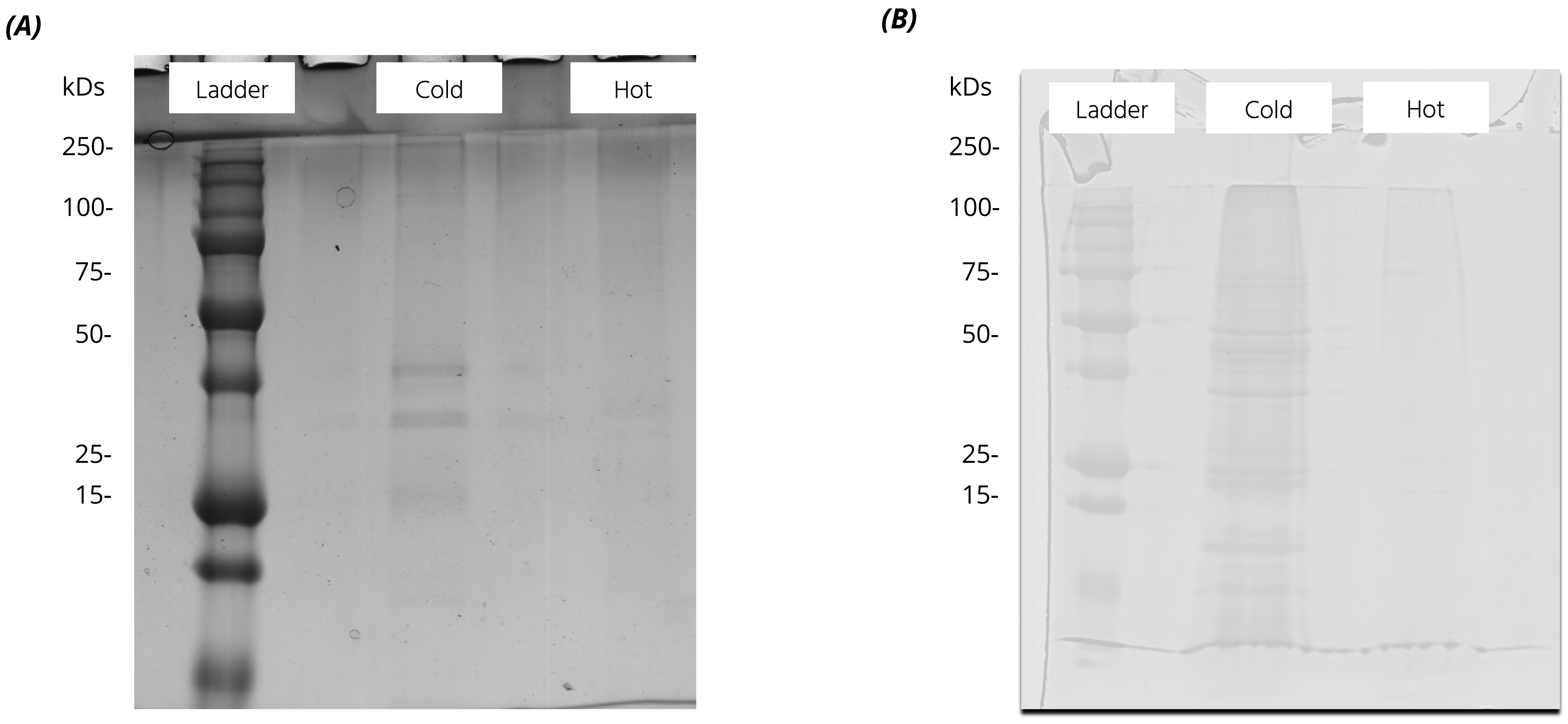


Figure 3- 1D-SDS-PAGE analysis of isolated samples through SEC, and maintained in vitro, fractionated by SDS-PAGE and stained with Coomassie blue. (A) Gel electrophoresis analysis of extracellular vesicles secreted from S. solidus when cultured in vitro at 4℃ (cold) and 39℃ (hot). (B) Gel electrophoresis analysis of extracellular vesicle depleted excretory secretory proteins secreted from S. solidus when cultured in vitro at 4℃ (cold) and 39℃ (hot).

GST Classes	GST hits to *S. solidus*		
	***F. gigantica*[6]**	***A. perfoliata*[7]**	**Total unconfirmed**
Mu	3	1	4
Sigma	2	3	3
Omega	1	TBC	1

Table 1- Homology searching of Glutathione transferases (GSTs) found in other known helminth secretomes such as Fasciola gigantica and Anoplocephala perfoliata against the secretome of Schistocephalus solidus. TBC- To be confirmed.

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