

Background

- 1). *Sepia esculenta*(*S. esculenta*) is mainly distributed in the western Pacific, along the coasts of China, southern Hokkaido, and the Philippines.
- 2). As cephalopod hard tissues do not always exhibit "one-day round", their microstructures should be validated before use in age determination.
- 3). It remains unclear which hard tissues of *S. esculenta* are suitable for reliable age determination.

Objective

- 1). We investigated the microstructures of the beak, cuttlebone, eye lenses, and statolith of *S. esculenta* to determine the optimal material for age estimation and to characterize its growth patterns.

Sampling

- 1). **Collection date and site:** Sep-Nov, 2021 (31-36° N, 119-122° E).
- 2). **Sample number:** A total of 360 *S. esculenta* samples were randomly collected and collected in the survey by Chinese fishing vessels.
- 3). **Sample preservation:** Beaks, eye lenses, and statoliths were preserved in 75% ethanol, while cuttlebones were sealed in plastic bags and stored at −20 °C.

Materials & methods

- 1). Preparation of *S. esculenta* hard tissues (beak, cuttlebone, eye lens, and statolith) involved sectioning, embedding, grinding, and polishing to expose growth increments for age and growth analysis.
- 2). Hard tissue sections of *S. esculenta* were imaged at appropriate magnifications and stitched in Photoshop for age estimation.
- 3). Relationships between growth increments and mantle length were modeled using AIC, and statistical analyses were performed in R, Excel, and SPSS.

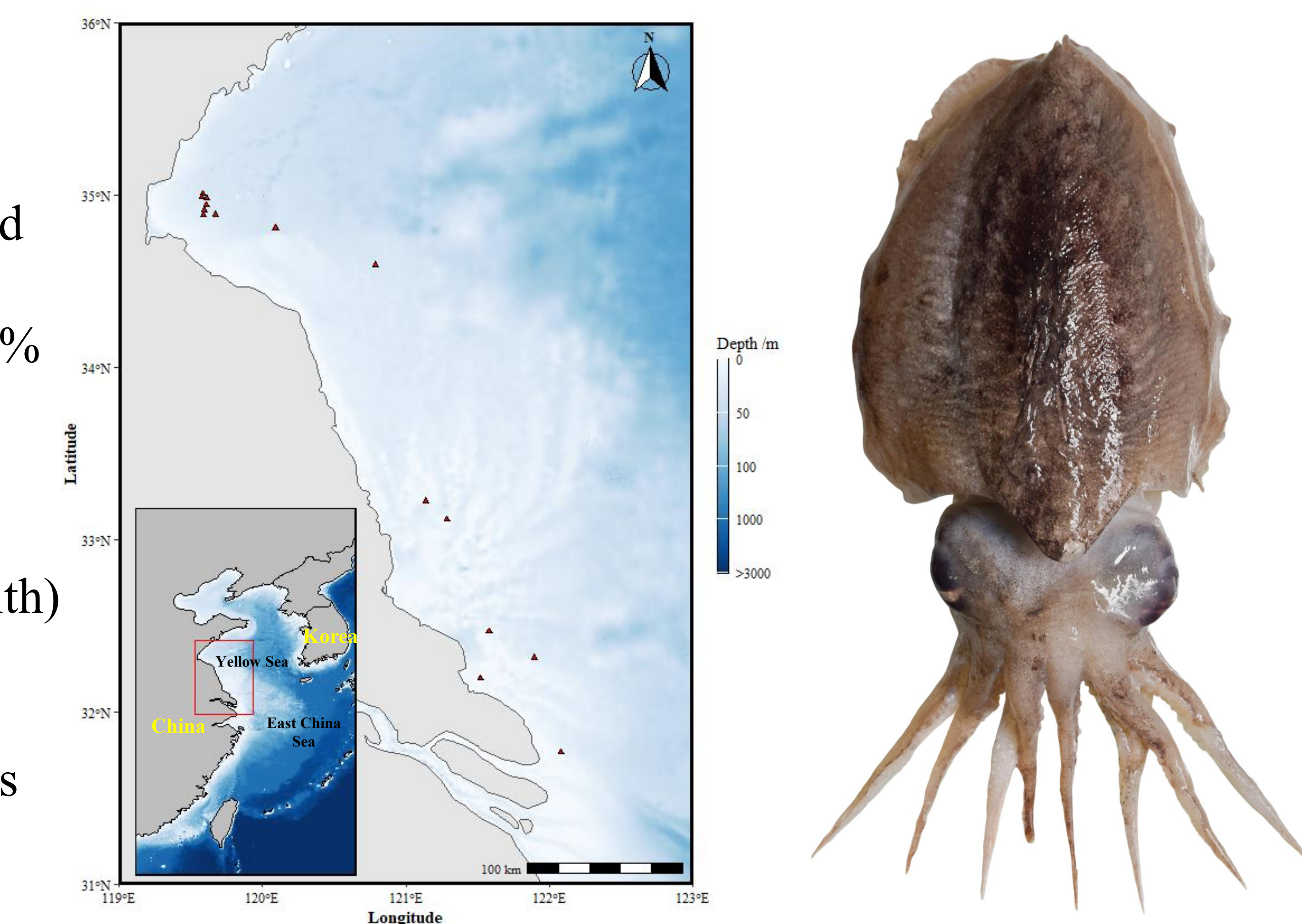


Fig.1 Sampling stations

Fig.2 *Sepia esculenta*

Results

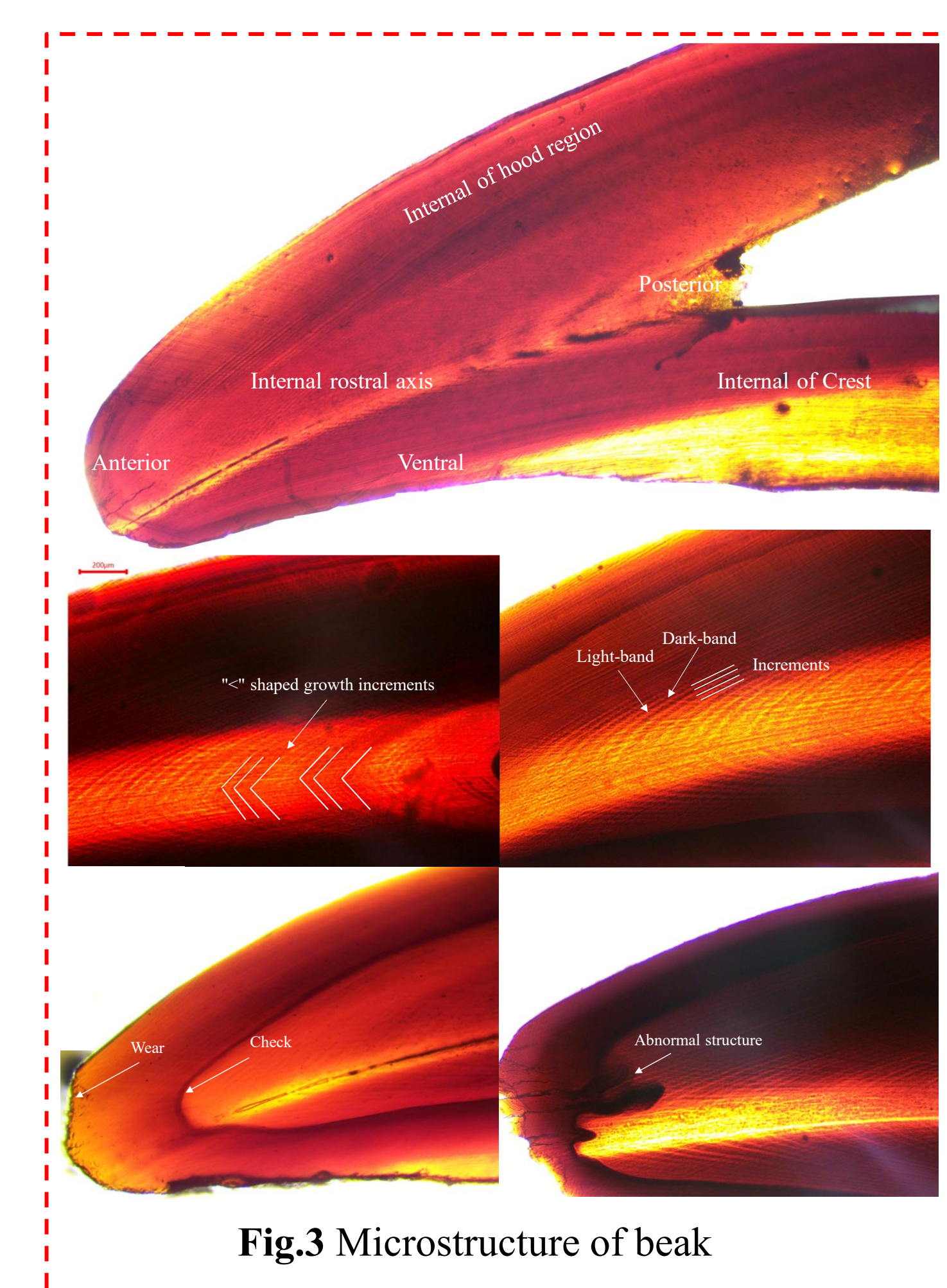


Fig.3 Microstructure of beak

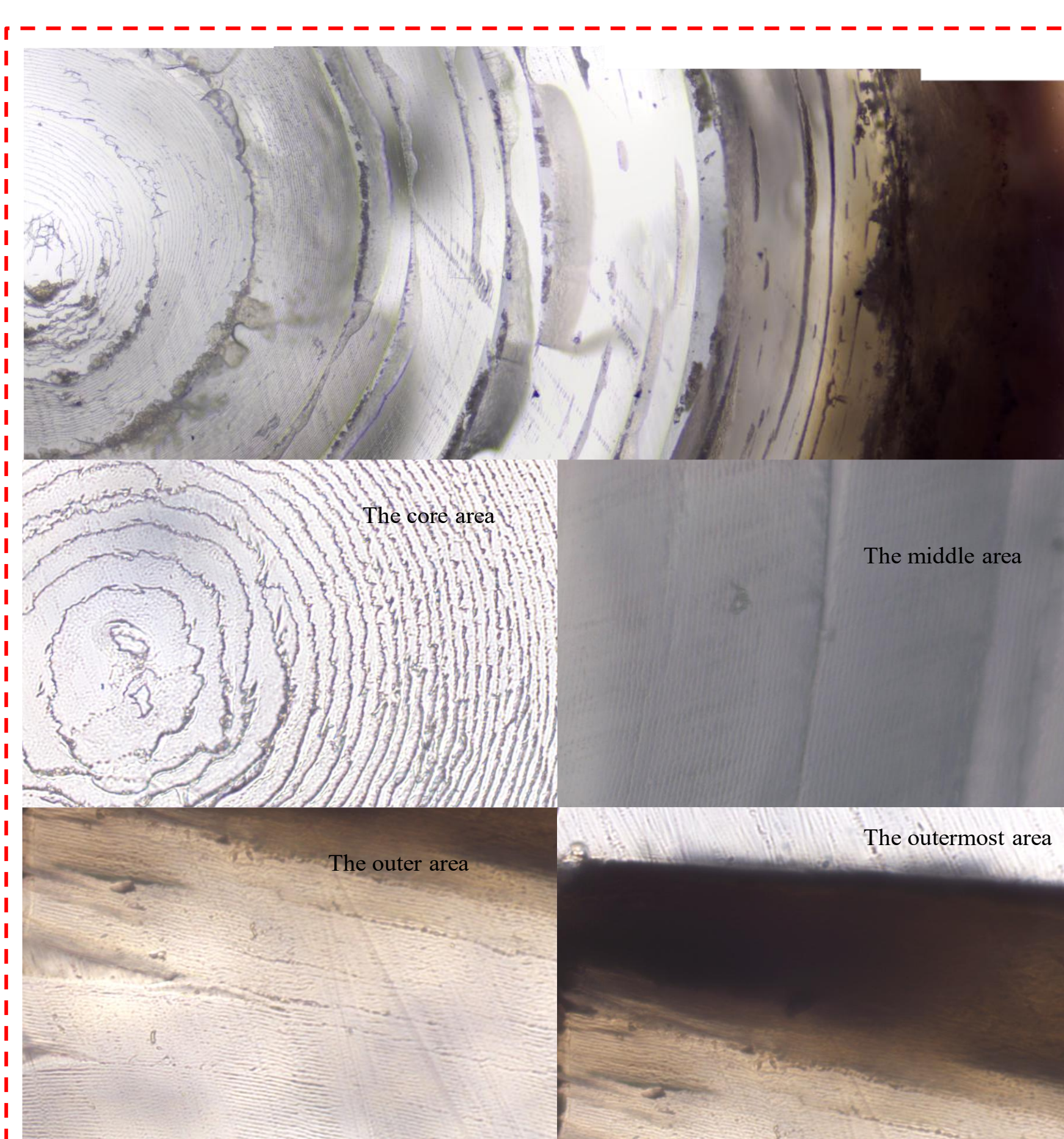


Fig.4 Microstructure of the eye crystal

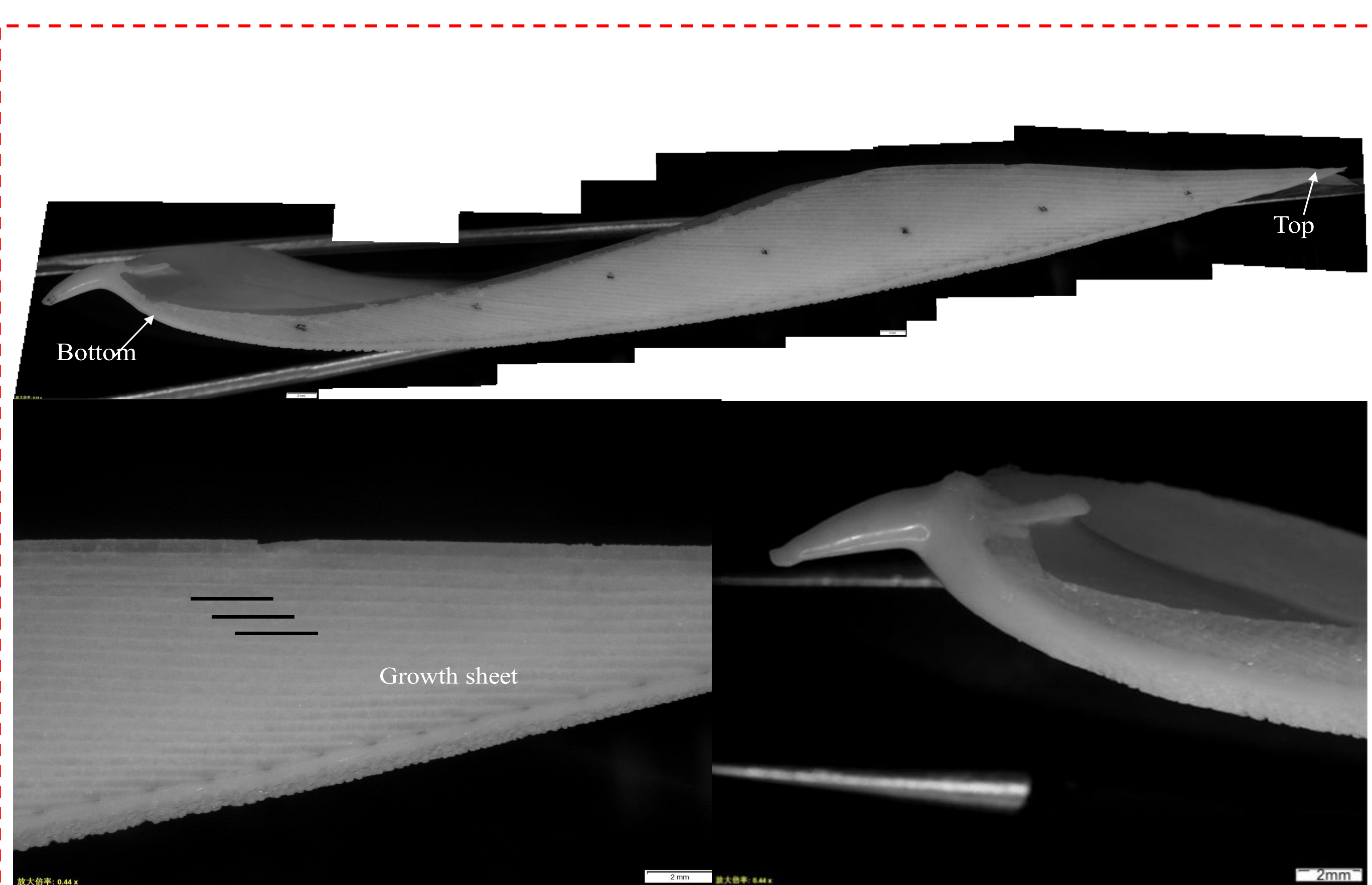


Fig.5 Microstructure of cuttlebone

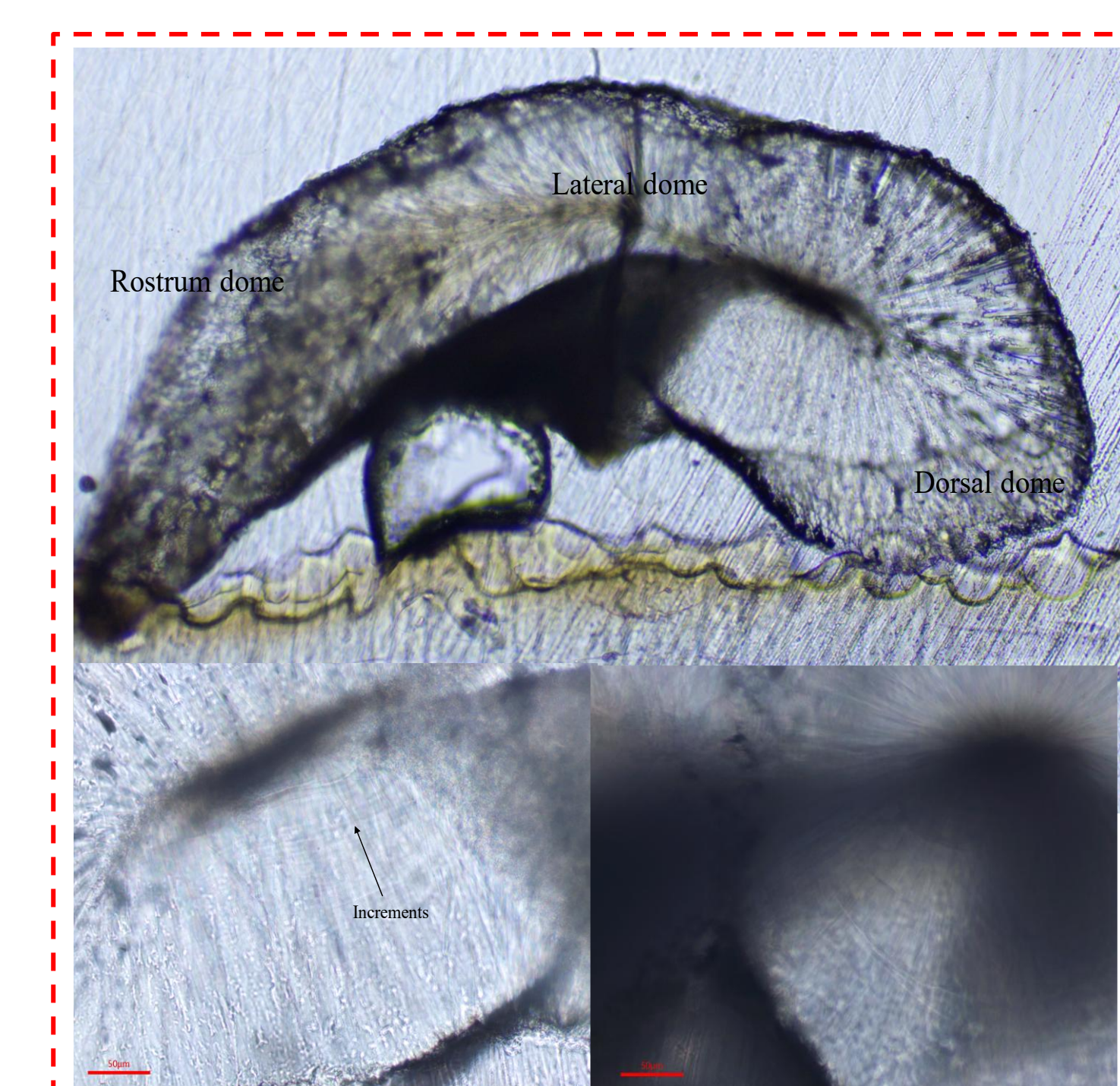


Fig.6 Microstructure of Statolith

Hard tissue	Number	Mantle length/mm	N
Beak	181	55~201	59~152
Cuttlebone	53	55~196	52~146
Eye crystal	51	74~201	375~1357
Statolith	60	55~201	-

Tab.1 Increments comparison of hard tissues

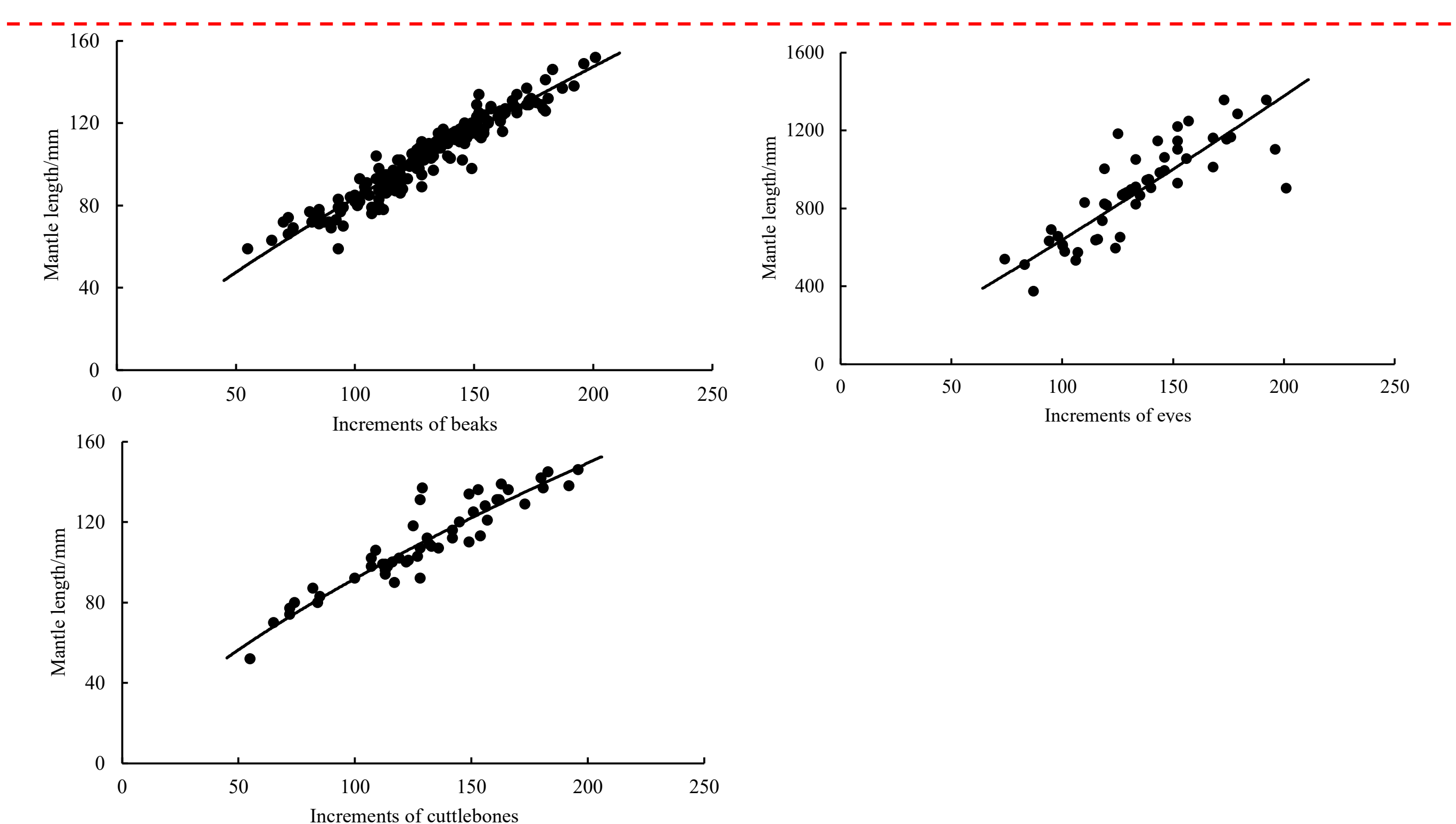


Fig.7 Relationship between mantle length and growth increments in the beak, cuttlebone, and eye crystal

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Conclusions

- 1). In *S. esculenta*, the beak provides the most reliable age marker with clear daily increments; the cuttlebone is supplementary, while the eye lens and statolith are unsuitable.
- 2). Except for the statolith, the growth increments in the beak, eye lens, and cuttlebone of *S. esculenta* can be used to characterize its growth patterns, and the number of increments in these structures shows a power function relationship with mantle length.

Acknowledgement

- 1). Thanks for squid samples and technology from my group and institution.
- 2). Thanks for China's Squid Fishing Technology Group.
- 3). Thanks for this presentation could be displayed in CIAC 2025.