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COMPARATIVE MORPHOLOGICAL STUDY OF OTOLITHS IN TAIWANESE ANGUILLIFORMES FISHES

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Key words: eel, sagitta, asteriscus, lapillus

ABSTRACT

Otoliths are calcareous structures found in the labyrinth membrane in the otic capsules of teleost fishes and are constituted of three pairs: the *sagittae*, *lapilli* and *asterisci*. They have long been recognized as being species specific. A comparative morphology of the sagittae, asterisci and lapilli among six eel species of Anguilliformes belonging to the Family Congridae, Muraenesocidae and Muraenidae was studied. A total of 64 specimens belonging to *Bathyconger wallacei* (n = 23), *Conger japonicus* (n = 9), *Muraenesox cinereus* (n = 10), *Echidna polyzona* (n = 4), *Gymnothorax eurostus* (n = 4) and *Gymnothorax reticularis* (n = 14) were collected from Tungkang, Chengkung, Changbin and Nanfangao, Taiwan, during the months of January and February 2013. The three pairs of otoliths were extracted from the otic capsules of the eels and observed by light and scanning electron microscopy. The morphology of the sagitta showed differences in shape among *B. wallacei, C. japonicus, M. cinereus* and *G. reticularis*, but similar shapes between *E. polyzona* and *G. eurostus*. While the morphology of the lapillus showed some similarities in shape among *B. wallacei* and *C. japonicus* and among *M.cinereus, E. polyzona, G. eurostus* and *G. reticularis*. The results also showed that the asteriscus is capable of conveying species specific information, with the asteriscus of each species varying in form. The three otolith types were the largest in *C. japonicus* and *M. cinereus* and the smallest in *E. polyzona, G. eurostus and G. reticularis*. The usage of the three pairs of otoliths allows for a more informative differentiation between the Anguilliformes species in Taiwan.

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I. INTRODUCTION

Otoliths are massive calcium carbonate structures found in the labyrinth membrane in the otic capsules of teleost fishes which have long been recognized as being species specific. They provide a sense of balance to fish in much the same way as that the inner ear provides in humans [12]. In teleost fishes the inner ear contains a labyrinth system which divides itself in two parts; each labyrinth then includes three semicircular canals or end organs which contain an otolith within an epithelial sac [21]. According to the respective end organ, the following three different otolith types can be distinguished: the sacule, the lagena and the utricle. Hence, each compartment contains an ear stone or otolith: the sagitta, astersicus and lapillus respectively [3, 15].

In fresh waters, we know only of the research on eel, *Anguilla anguilla*, in the estuary of the Guadalquivir river [6] who used the three otolith types. Comparative morphological study of the sagittal otoliths was only performed in deep-sea snipe eel, sawtooth eel and pelican eel that belong to the family Nemichthyidae, Serrivomeridae and Eurypharyngidae respectively [9]. Therefore, this study focused on Anguilliformes fishes found in the waters of Taiwan specifically six species that belong to the families Congridae, Muraenesocidae and Muraenidae. The Congridae (conger eels) are one of the most abundant and diverse families that can be found worldwide in tropical and sub-tropical latitudes; they are usually small with moderately large pectoral fins and eyes and with a slightly protruding upper jaw [17]. The Muraenesocidae (pike congers) can also be found inhabiting tropical and sub-tropical waters, including the Mediterranean. However, eels of this family are heavy-bodied predatory eels with strong teeth and jaws hence this family includes some of the largest of all eels [18]. On the other hand, the Muraenidae (moray eels) are perhaps the easiest to recognize due to their large mouth, small gill opening and absence of their pectoral and pelvic fin and just like the Congridae, these eels can also be found in all tropical and sub-tropical oceans and seas [5].

Due to the lack of comparative morphological studies that utilize the three otolith types, this study would aid to determine any morphological difference among the three otolith

Fig. 1. The six Anguilliformes species that were used for the otolith study in Taiwan. A, *Bathycongrus wallacei*; B, *Conger japonicus*; C, Muraenesox cinereus; D, *Echidna polyzona*; E, *Gymnothorax eurostus*; F, *Gymnothorax reticularis*.

types of the six eel species of Anguilliformes that belong to the family Congridae, Muraenesocidae and Muraenidae.

II. MATERIALS AND METHODS

Eels were collected from Tungkang (22° 28'N, 120°26'E), Chengkung (23°9'N, 121°25'E), Changbin (23°18'N, 121°29'E) and Nanfangao (24°35'N, 121°52'E) fish market during the months of January and February 2013. A total of 64 specimens were collected belonging to, *Bathyconger wallacei* (n = 23), *Conger japonicus* (n = 9), *Muraenesox cinereus* (n = 10), *Echidna polyzona* (n = 4), *Gymnothorax eurostus* (n = 4) and *Gymnothorax reticularis* (n = 14) (Fig. 1). Eels were identified by the method of Hatooka [8] and Smith [19], and then measured using the terminology and method presented by Böhlke [4]. The eels were labeled with their perspective catalog number. An Olympus SZ61 dissecting stereo microscope was used to extract the three pairs of otoliths. The method of removal was taken from Homayuni *et al.* [10] with some modification: the otoliths were removed by turning the ventral side of the eel upward to allow a complete removal of the lower jaw in order to expose the base of the skull. The otoliths were then washed with distilled water and placed in plastic vials. The otoliths were air dried, and then an Olympus SZX16 was used to take photographs of the medial faces of only the right otoliths and these were placed against a black background in order to get a clear outline. For the SEM, the three pairs of otoliths with their medial face facing upwards

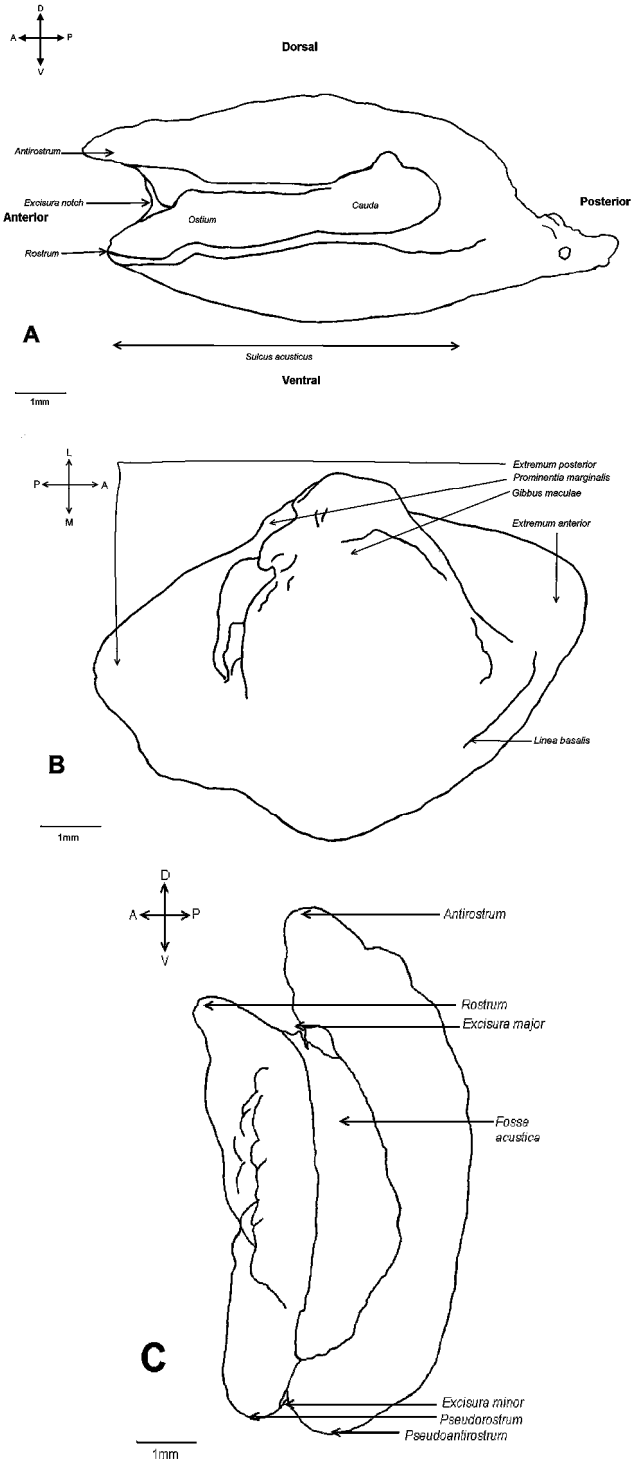

Fig. 2. The terminologies and positions for the features displayed by the otoliths, right otoliths are represented. D, V, A, P, L, and M represent dorsal, ventral, anterior, posterior, lateral (distal) and medial (proximal) directions respectively. A, *Sagitta*; B, *Lapillus*; C, *Asteriscus*.

were placed on aluminum stubs and sputter coated with platinum (Hitachi E-1010 ion sputter) for 30 seconds and then photographed using the Hitachi S-3400N Scanning electron microscope from National Taiwan Ocean University. The otoliths described were made on the basis of observation through the light and scanning electron microscopy photographs. The right sagittal otoliths were described using the terminology of Smale *et al*. [16] and Tuset *et al.* [22] while the right asteriscus and right lapillus were described using the terminology of Assis [1, 2] (Fig. 2).

III. RESULTS

1. Morphological Description of the Sagitta

1) Congridae

Bathyconger wallacei (Fig. 3A)

The anterior and posterior margin is round to oblique. There is no excisura major, no rostrum and no antirostrum on the anterior margin. There is no excisura minor, no postrostrum and no postantirostrum on the posterior margin. The dorsal and ventral margin is entire. The overall shape is oval. The sulcus is mesial and median. The ostium and cauda are peaked and slightly round.

Conger japonicus (Fig. 3B)

The anterior margin and posterior margin is slightly peaked and round. There is no excisura major, no prominent rostrum and no antirostrum present in the anterior margin. There is no excisura minor, no postrostrum and no postantirostrum present in the posterior margin. The dorsal margin is somewhat sinuate and the ventral margin is entire. Overall shape is spindle. The sulcus is ostial (opens onto the anterior-dorsal margin) and inframedian (the ventral area is noticeably smaller than the dorsal area). The ostium has a funnel-like shape, while the cauda is round-oval.

2) Muraenesocidae

Muraenesox cinereus (Fig. 3C)

The anterior margin and posterior margin are entire and round. There is no excisura major, no prominent rostrum and no antirostrum in the anterior margin. There is no excisura minor, no prominent postrostrum and no postantirostrum in the posterior margin. The dorsal margin is slightly crenate and the ventral margin is entire and round. Overall shape is oblong. The sulcus is ostial and inframedian. The ostium has a funnel-like shape, while the cauda is slightly round-oval.

3) Muraenidae

Echidna polyzona (Fig. 3D)

The anterior margin shows the presence of a slightly longer and wide rostrum and a smaller and peaked antirostrum; this presents the formation of a shallow and wide excisura major. The posterior margin is peaked and has no postrostrum, no postantirostrum and no excisura minor. The dorsal and ventral margins are entire. The overall shape is elliptic. The

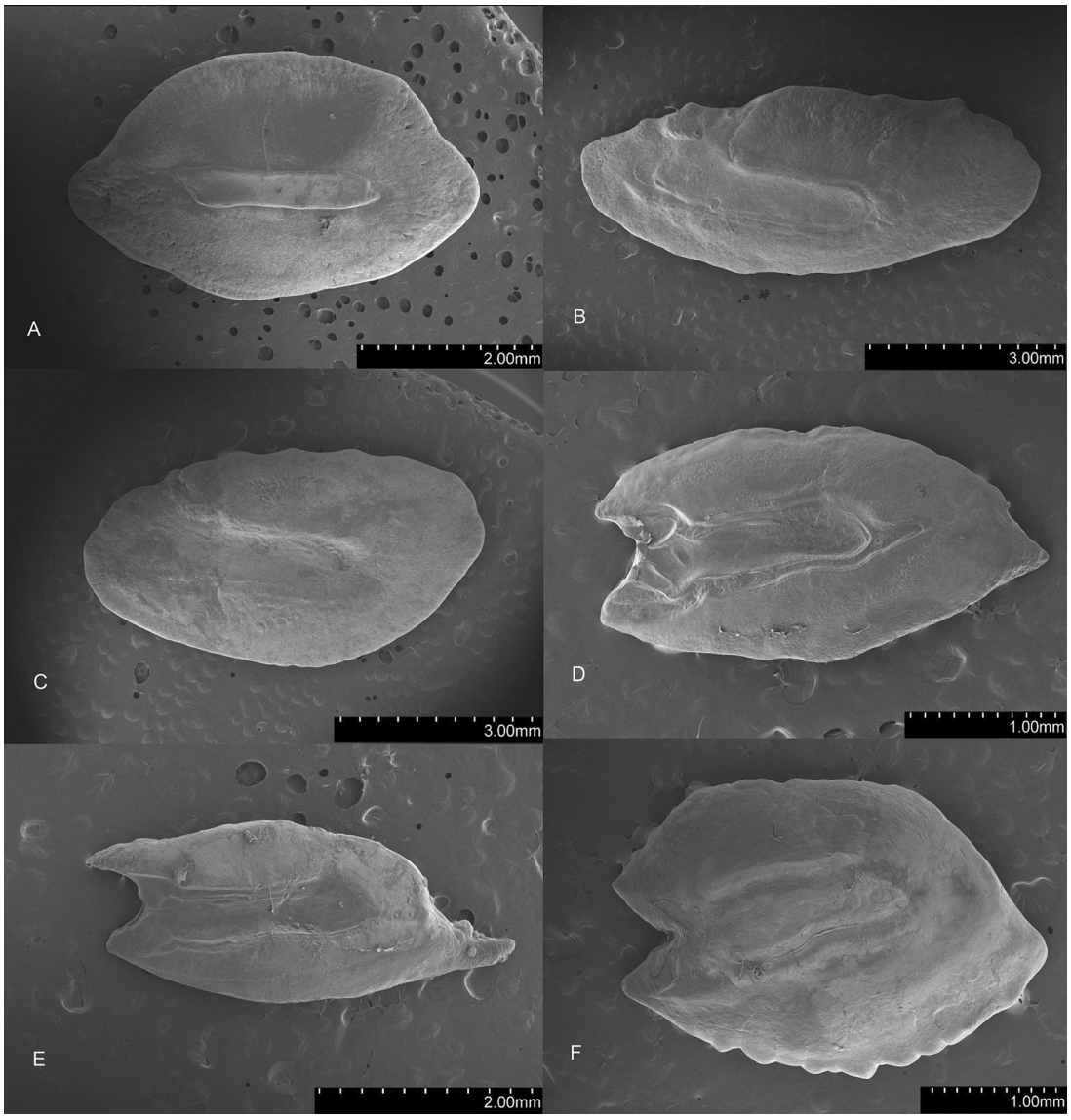

Fig. 3. The SEM photographs of the ventral view of the right sagitta of the six eel species: A, *Bathycongrus wallacei*; B, *Conger japonicus*; C, *Muraenesox cinereus*; D, *Echidna polyzona*; E, *Gymnothorax eurostus*; F, *Gymnothorax reticularis*.

sulcus is ostial and median. The ostium has a funnel-like shape, while the cauda is slightly round-oval.

Gymnothorax eurostus (Fig. 3E)

The anterior margin shows the presence of a blunt rostrum and a peaked antirostrum with the formation of a wide and slightly deep excisura major. The posterior margin is peaked and has no postrostrum, no postantirostrum and no excisura minor. The dorsal and ventral margins are entire. The overall shape is elliptic. The sulcus is ostial and median. The ostium has a funnel-like shape, while the cauda is round-oval.

Gymnothorax reticularis (Fig. 3F)

The anterior margin shows a well-developed excisura major and a prominent rostrum and antirostrum. The posterior margin exhibits a peaked end but no excisura minor so no postrostrum and postantirostrum is shown. The dorsal margin is entire and the ventral margin is serrate. Overall shape is oval. The sulcus opening is ostial and supramedian (the ventral area is noticeably larger than the dorsal area). The ostium has a funnel-like shape, while the cauda is round-oval.

2. Morphological Description of the Lapillus

1) Congridae

Bathyconger wallacei (Fig. 4A)

The ventral view shows the margins are entire. The Gibbus maculae (a protuberance on the otolith) is in the middle, does not protrude outward and it is angulous. The linea basalis can be seen. There is no prominentia marginalis. The extremum posterior and anterior are round and both have the same size.

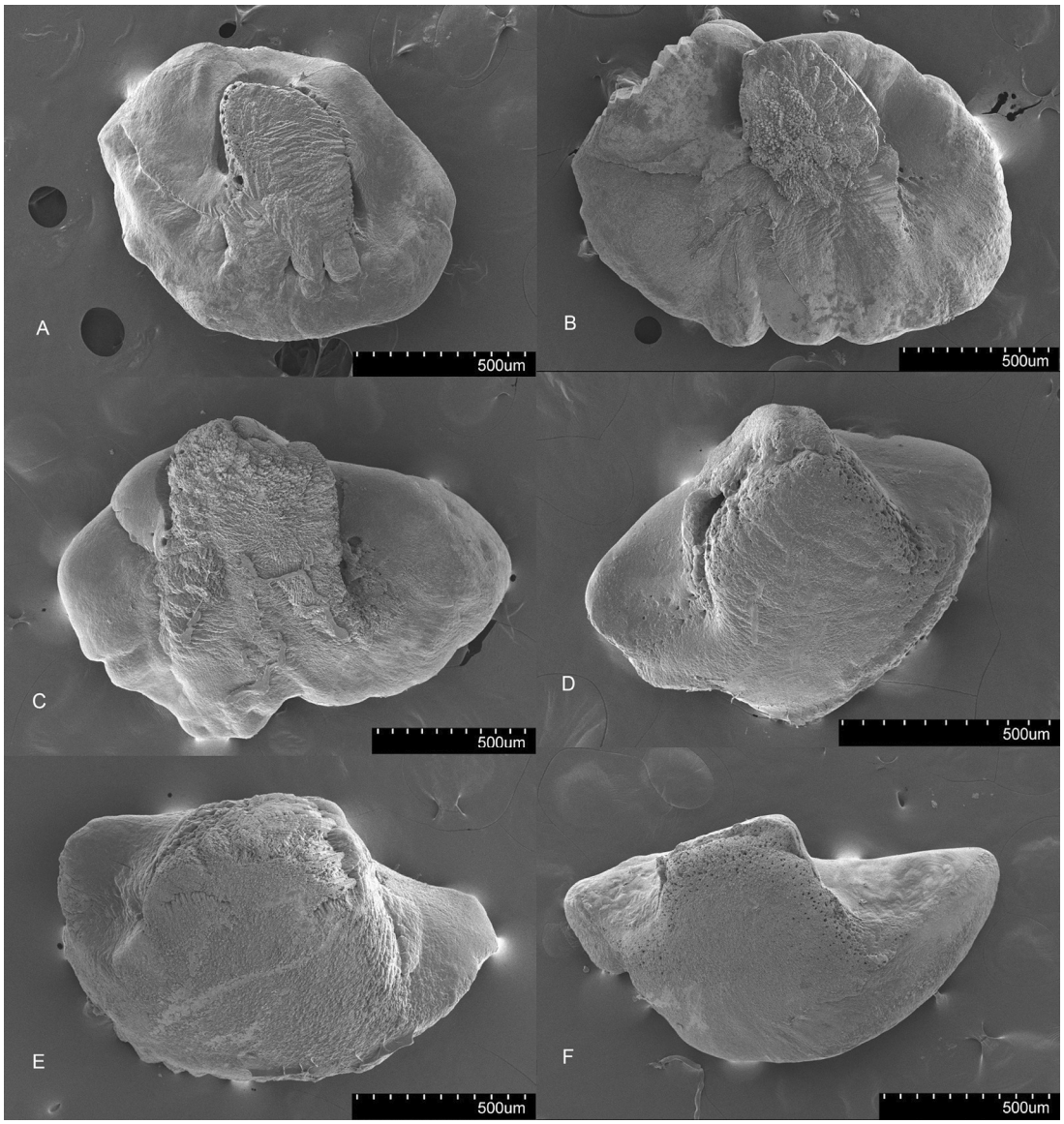

Fig. 4. The SEM photographs of the ventral view of the right lapillus of the six eel species: A, *Bathycongrus wallacei*; B, *Conger japonicus*; C, *Muraenesox cinereus*; D, *Echidna polyzona*; E, *Gymnothorax eurostus*; F, *Gymnothorax reticularis*.

Conger japonicus (Fig. 4B)

The ventral view shows the margins crenate. The Gibbus maculae faces the lateral margin; it is small and angulous in shape. There is no linea basalis and no prominentia marginalis. The extremum anterior and posterior are round but the extremum anterior has a smaller space.

2) Muraenesocidae

Muraenesox cinereus (Fig. 4C)

The ventral view shows the margins crenate and lobed. The Gibbus maculae protrudes outward on the lateral side and is large and squared. The linea basalis can be somewhat distinguished. The prominentia marginalis cannot be seen. The extremum posterior and anterior is round but the extremum posterior is smaller.

3) Muraenidae

Echidna polyzona (Fig. 4D)

The ventral view shows the margin crenate. The Gibbus maculae is large and angulous. The linea basalis can be slightly seen. The prominentia marginalis can be seen. The extremum posterior and anterior is round but the extremum posterior is larger.

Gymnothorax eurostus (Fig. 4E)

The ventral view shows the margins being serrate. The Gibbus maculae is large and globose. There is no linea basalis and no prominentia marginalis. The extremum posterior and extremum anterior are round and have the same size.

Gymnothorax reticularis (Fig. 4F)

The ventral view shows the margins are entire. The Gibbus maculae faces the lateral margin; it is large and globose in shape. There is no linea basalis and no prominentia marginalis. The extremum posterior is slightly blunt and smaller than the extremum anterior which is slightly pointed and longer.

3. Morphological Description of the Asteriscus

1) Congridae

Bathyconger wallacei (Fig. 5A)

The fossa acustica is convex and is thin and shallow. The lobus minor is smaller and bulged. The lobus major is larger and flat. The campus major is convex. The campus minor is concave. There is a pointed rostrum and a round antirostrum. No excisura major forms. There is no postrostrum, no postantirostrum and no excisura minor. The ventral side is round.

Conger japonicus (Fig. 5B)

The fossa acustica is narrow. The lobus minor is smaller than the lobus major. The campus major is concave while the campus minor is convex. The posterior side (lobus major) has seven protuberances.

2) Muraenesocidae

Muraenesox cinereus (Fig. 5C)

The fossa acustica is wide and deep. The lobus minor and lobus major are about the same size and both are bulged. The campus major is convex while the campus minor is concave. The presence of the rostrum and antirostrum can be seen, they are both peaked. The excisura major can also be seen having a V-shape. The pseudorostrum and pseudoantirostrum can also be seen and both are circular which allows a slight development of the excisura minor.

3) Muraenidae

Echidna polyzona (Fig. 5D)

The fossa acustica is not clearly differentiated. The lobus major is small and lobus minor is large. The campus minor is very wide and convex and the campus minor is wide and convex but smaller than the campus minor. The rostrum is big and round and the antirostrum is small and round. The excisura major is deep and U-shaped. There is no postrostrum, no postantirostrum and no excisura minor. The ventral side is bulged and has a round peak.

Gymnothorax eurostus (Fig. 5E)

The fossa acustica is wide (oval-like) and deep (hollow-like). The lobus minor and lobus major are the same size. The campus major and campus minor are convex. There is no rostrum, no antirostrum, no excisura major, no pseudorostrum, no pseudoantirostrum and no excisura minor. There is a peak that forms on the dorsal and ventral side.

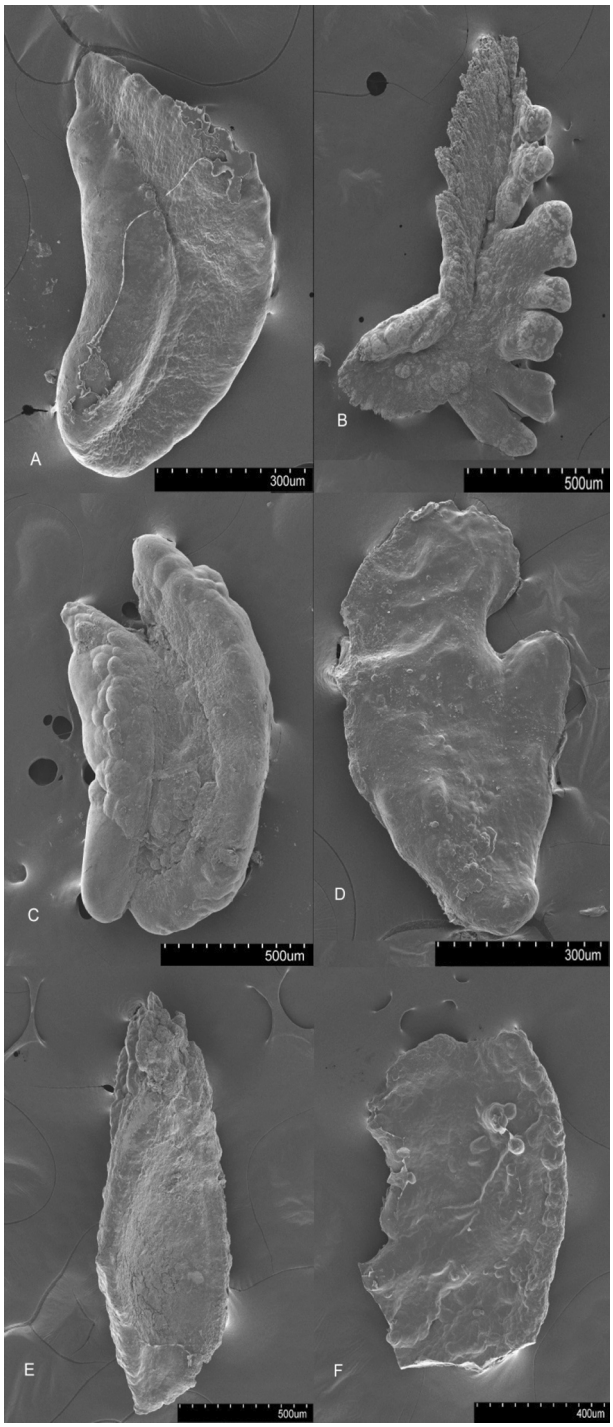

Fig. 5. The SEM photographs of the ventral view of the right asteriscus of the six eel species: A, *Bathycongrus wallacei*; B, *Conger japonicus*; C, *Muraenesox cinereus*; D, *Echidna polyzona*; E, *Gymnothorax eurostus*; F, *Gymnothorax reticularis*.

Gymnothorax reticularis (Fig. 5F)

The fossa acustica is very wide and not clearly differenti-

ated. The lobus minor is smaller than the lobus major. The campus major is concave while the campus minor is convex. It is flat and thin.

IV. DISCUSSION

In this study it was observed that the sagitta of the *Bathyconger wallacei* has an oval shape and is the only that presents its sulcus acusticus mesial and median. This is in contrast to *Conger japonicus* which is spindle in shape but the sulcus acusticus is ostial and inframedian. Lin and Chang [13] in their "Otolith Atlas of Taiwan Fishes" described *Muraenesox cinereus* as being oblong in shape and median; however, it slightly differs with the results in this study, because *M. cinereus* presents the sulcus acusticus as inframedian but the shape is still the same. The sagitta of *Echidna polyzona* is elliptic and its sulcus acusticus is median, though, it is also noted that the sagitta of *Gymnothorax eurostus* is also elliptic and its sulcus acusticus is median; both of them present deep sulcus acusticus. However, these two species differ because the sagitta of *G. eurostus* presents a pointed antirostrum and on its posterior (ventral) it has a very long peak, as compared to *E. polyzona* that has its rostrum longer than its antirostrum and on its posterior side it presents a small peak. Therefore, the result obtained in this study is not in agreement with Homayuni *et al*. [10] who stated that studies on fossil and extant otoliths have demonstrated that the sulcus morphology usually is consistent among the species of a single genus and thus this feature is likely controlled genetically. However, *E. polyzona* and *G. eurostus* are both found in similar niche's, Chengkung and Changbin respectively, which agrees with Zorica *et al*. [24] who indicated that fish which occupy the same ecological niche show resemblances in otolith shape. What's more is that *Gymnothorax reticularis*, differs from *Gymnothorax eurostus* which is under the same genus, due to it having an oval shape with its sulcus acusticus being supramedian which is in accordance to the description that was given by Lin and Chang [13]. Therefore, as can be seen in most atlas of otoliths structure, the shape of the saccular otoliths vary greatly, and although some are simple ellipsoids, others have highly complex patterns with various projections and invaginations that are species specific [14] which can also be seen in this study.

Assis [2] stated that the lapilli are morphologically rather homogenous among most fish groups and this concurs with the results that were obtained for the lapillus of the species in this study. The lapilli of *Conger japonicus* and *Bathyconger wallacei* both have a discoid shape, as compared to *Muraenesox cinereus*, *Gymnothorax eurostus*, *Echidna polyzona* and *Gymnothorax reticularis* which present an oval shape. The lapilli are composed of a wedge-shaped dorsal body, narrowing from the lateral to the medial side, below which occurs a more or less voluminous protuberance, the gibbus maculae [2]. This gibbus maculae has a slight variation in shape and size among the six eel species, with *C. japonicus* being small and angulous, while in *G. reticularis* it is large and globose so it protrudes outwards. In *M. cinereus*, however, this protuberance seems to be squared and compressed which also differs with *G. eurostus* which has its gibbus maculae globose and bulky. *E. polyzona* tends to have the same description as *G. eurostus*, however, the only difference is that the prominentia marginalis can be seen in *E. polyzona*. The only species that does not have its gibbus maculae bulky and protruding outward is *B. wallacei;* this species has it angulous and flattened. The results obtained agree that among the three pairs of otoliths, the lapilli are the ones that have the most regular shape, the most homogenous constitution in all fish taxa and the fewest number of usable diagnostic features [2].

In this study it was illustrated that the asteriscus is not homogenous among fish taxa, as the lapillus is. The asteriscus represents a very small fraction of the total diversity of forms that it displays and they are far from being morphologically homogenous among the fish taxa [1]. The asteriscus all vary in shape and fossa acustica among the six eel species and because of their unique form it allows them to be distinguished from each other. This can be noted in *C. japonicus* which has seven protuberances on its posterior side and the fossa acustica which is very thin, but *G. reticularis* does not show these characteristics. *G. reticularis* is thin and the only characteristic observed is the lobus major which tends to slightly influence the formation of a fossa acustica which is not clearly developed. These two species start to show a great variation, however when looking at the asteriscus of *M. cinereus* then it can be noted the different developments that each asteriscus goes through. In this study, the asteriscus of *M. cinereus* presents a highly developed otolith by clearly showing the deep and wide fossa acustica with its two bulged lobes (major and minor) and because of these features it also shows two peaks on its dorsal side and ventral side. The asteriscus of *M. cinereus* shows more features than any other asteriscus in this study. *G. eurostus*, presents a canoe shape, the fossa acustica in the middle is deep and wide and the lobus major and minor are convex thus surrounding the fossa acustica. This is very different when comparing it to *E. polyzona*, because it has a rostrum that is even larger than the antirostrum and the fossa acustica is slightly exhibited. These characteristics can also be seen in *B. wallacei* and they are also different because it has its fossa acustica thin and shallow and curves in a convex manner. The comparison of the asteriscus between the species shows that there is a greater variation in shape and presence of characteristics, therefore it is frequently easy to visually confirm that they are considerably different and that the existence of a common morphology among them is evident [1, 7].

What's more is that the size of the otolith is also a character that is important for knowing a species, since otoliths vary dramatically in size between different species [12]. The otoliths of the species in this study have different ranges in size, with *Conger japonicus* and *Muraenesox cinereus* presenting

the highest range within the three otolith types being 6.37 mm-8.70 mm, 0.95 mm-1.29 mm and 1.17 mm-1.60 mm in length for saggita, lapillus and asteriscus respectively. It is known that the growth of the otolith is influenced by various factors such as seasonal variations, temperature, habitat and diet [10, 20, 23] which is why in this study it was noted that *Echidna polyzona*, *Gymnothorax reticularis* and *Gymnothorax eurostus* had similar ranges for the three otolith types being 2.68 mm-4.35 mm, 0.82 mm-1.27 mm and 0.22 mm-0.49 mm for sagitta, lapillus and asteriscus respectively since *E. polyzona* and *G. eurostus* share the same habitat range, while *G. reticularis* shares the same diet. While *Bathyconger wallacei* was at the intermediate level, with 2.55 mm-8.27 mm, 0.75 mm-1.51 mm and 0.31-0.99 mm for sagitta, lapillus and asteriscus respectively. The size range for the sagitta in all the species is larger than the lapillus and asteriscus, hence, due to their large size which makes it easily accessible and degree of inter-specific variation it is the most widely used tool in comparative studies [11, 15, 22]. While the size ranges shows the lapillus to be the second largest and the asteriscus the smallest, however, only few studies have investigated inter- and intra-specific shape variation of these two otolith types [1, 2, 15]. The results in this study has allowed the usage of the three otolith types and has resulted in a much better separation and thus suggests that the combination of the sagitta, lapillus and asteriscus presents better information than just analyzing one otolith type.

V. CONCLUSION

The morphological comparison done in this study allowed the usage of the three otolith types: the sagitta, asteriscus and lapillus which are useful in adding to the basic information about the six eel species: *Bathyconger wallacei*, *Conger japonicus*, *Muraenesox cinereus*, *Echidna polyzona*, *Gymnothorax eurostus* and *Gymnothorax reticularis*. This adds to the general information about Anguilliformes in Taiwan. Further studies should be conducted in order to analyze more eel species using the three otoliths from different Anguilliformes families in order to understand its morphological and taxonomical relationship.

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