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Short Communication

Development of free neuromasts in *Engraulis ringens* and *Strangomera bentincki* (Teleostei, Clupeiformes) early larvae

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ABSTRACT. Neuromast morphology and distribution are characterized during early ontogeny of anchoveta (*Engraulis ringens*) and common sardine (*Strangomera bentincki*). Although both species share morphological features, they show several differences during their early ontogeny, such as size at hatching and yolk absorption. Larvae were obtained from incubation of planktonic eggs (at 12°C), collected during the spawning season 2001 (August-September) at Coliumo Bay. The neuromasts were observed from hatching to 25 days, and the pattern of neuromast appearance, in newly hatched larva, until yolk absorption, was determined using Janus Green staining and scanning electronic microscope. Results showed a similar pattern of neuromast development in both species. At hatching, two pairs of neuromasts were observed in the cephalic area and 8-9 in the rest of the body, which increased to 19 pairs and to 30-39 pairs at a larval size of 11 mm, respectively. On the average, 12 hair cells per neuromast were counted, with little variation among neuromasts. The polarity of these hair cells was closest to multiple polarity. Neuromast positioning for both species, anchoveta and common sardine larvae, are similar to those of *Engraulis mordax* and *Clupea harengus*, respectively. The similar development pattern of these species seems to be related to similar functional constraints and close taxonomic affinity.

Keywords: neuromasts, larvae, *Engraulis*, *Strangomera*, Clupeiforms, Chile.

Desarrollo de neuromastos libres en larvas tempranas de *Engraulis ringens* y *Strangomera bentincki* (Teleostei, Clupeiformes)

RESUMEN. Se caracteriza la distribución y morfología de los neuromastos en la ontogenia temprana de la anchoveta (*Engraulis ringens*) y sardina común (*Strangomera bentincki*). Aunque ambas especies comparten varias características morfológicas, muestran diferencias durante la ontogenia temprana, tales como tamaño a la eclosión y absorción de vitelo. Las larvas fueron obtenidas desde la incubación de huevos planctónicos a 12°C, recolectados en la bahía Coliumo durante la estación reproductiva 2001 (agosto-septiembre). Los neuromastos fueron observados desde la eclosión hasta los 25 días posteriores, usando la tinción vital de verde de Jano y microscopía electrónica de barrido. Los resultados muestran un patrón similar de desarrollo en ambas especies. A la eclosión se observaron 2 pares de neuromastos en la región cefálica y entre 8 y 9 en el resto del cuerpo, que a la longitud de 11 mm aumentan a 19 y entre 30 y 39 pares respectivamente. En promedio, se contabilizaron 12 células ciliadas por neuromasto con escasa variación entre neuromastos, con una polaridad cercana a la polaridad múltiple. La posición de los neuromastos de ambas especies, anchoveta y sardina, fueron similares a las descritas para *Engraulis mordax* y *Clupea harengus*, respectivamente. El patrón similar de desarrollo de estas especies parece relacionado con sus restricciones funcionales y estrecha afinidad taxonómica.

Palabras clave: neuromastos, larvas, *Engraulis*, *Strangomera*, Clupeiformes, Chile.

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Neuromasts are mechanical receptors of anamniote aquatic vertebrates whose function is to detect vibrations in the surrounding water. In fishes they are involved in various functions such as predator evasion, group formation, and prey capture (Bone *et al.*, 1995). Most Teleostei hatch with a set of functional, or nearly functional neuromasts on the head and body (Webb, 1999). These sensory organs develop as free neuromasts early in the ontogeny from anterior (preotic) and posterior (postotic) placodes (Northcutt, 2003), and may become enclosed inside canals during larval and juvenile development (O'Connell, 1981).

Each neuromast is composed of several hair cells that contain a bundle of stereocilia and a single kinocilium. These projections are embedded in a long, prominent gelatinous cupula that grows continuously (Blaxter, 1984). Each cell is directionally sensitive, with the polarity determined by the relative position of kinocilia and stereocilia (Webb, 1999). The number, form, and size of the neuromasts (as well as the number of hair cells) increase during the ontogeny, which suggests functional changes through development. The pattern of neuromast development is affected by the relative importance of mechanical perception (*e.g.*, Blaxter & Fuiman, 1990; Mukai *et al.*, 1994), in particular, the onset of vision and other sensory systems, which differs between species (*i.e.*, larvae with compara-tively more developed eyes or olfactory organs tend to show slower neuromast development).

The anchoveta, *Engraulis ringens* Jenyns (Engraulidae), and common sardine *Strangomera bentincki* (Norman) (Clupeidae), are sympatric species that inhabit the Humboldt Current System. Both species have planktonic eggs and share similar morphological larval features such as pigmentation and body shape (Orellana & Balbontín, 1983). However, the two species differ in size at hatching (common sardine 2.5-3.2 mm and anchoveta 2.1-3.1 mm) and yolk absorption/eye pigmentation (common sardine 5.1-5.3 mm and anchoveta 3.8-4.0 mm; Herrera *et al.*, 1987). The information on their dietary composition suggests distinct capture abilities and possibly different perception abilities; common sardine larvae can capture more mobile prey than anchoveta larvae (Llanos-Rivera *et al.*, 2004). However, there is no information about neuromast in the larvae of these two species, except for the observation of small mechano-receptor protuberances located along the sides of the body in larval anchoveta by Fischer (1958). A comparative analysis of neuromast development between these two species, which share a similar larval morphology, might help to explain the observed ontogenetic differences. The hypothesis that early larvae of *S. bentincki* develop a more complex neuromast system for mechanical perception to feed on mobile prey will be tested.

In this study, the development of free neuromasts during the early ontogeny (hatching to early post-flexion) was characterized in anchoveta and common sardine, in terms of number, position, sequence of appearance, length of cupulae, number of hair cells, and polarity. These results are then compared with related and unrelated species.

Larvae were obtained from the incubation of eggs collected from Coliumo Bay, Chile (36º32'S, 72º56'W). Eggs were incubated at 12°C in filtered (0.5 mm) and sterilized (UV) seawater. Post-yolk sac larvae were fed with micro-algae, rotifers, and *Artemia* nauplii sequentially.

In order to identify the neuromast appearance patterns throughout the yolk-sac period, anesthetized (benzocaine, BZ-20) larvae were stained in 0.1% Janus Green solution in sea water (Blaxter, 1984). This procedure was used to obtain information on cupulae length and neuromast number and position. For a more detailed structural characterization, scanning electron microscopy (SEM) was used. Larvae from yolk sac to post-flexion were anesthetized, measured, preserved in 2.5% glutaraldehyde, prepared for SEM observation following Elston (1981), and subsequently observed from the left and right sides. This procedure was used to obtain information on distribution and the number of neuromasts as well as hair cell polarity.

To assess differences in the length of the cupula and the rate of neuromasts proliferation between species, a t-test and ANCOVA were carried out respectively. In these analyses the software Statistica 7.0 was used.

Anchoveta and common sardine hatched with a complement of two cephalic pairs of neuromasts anterior to the otic capsules, plus 7-8 pairs on the trunk (Fig. 1a). The first to develop corresponds to the otic neuromast (from the preotic series), also observed in other clupeiforms such as *Alosa sapidissima* (Shardo, 1996). This pair was observed to develop before hatching in anchoveta (stage XI of Moser & Ahlstrom, 1985). The second pair develops anteriorly on the snout; with the cupulae projecting forward (Figs. 1b, 2).

At hatching, both species have trunk neuromasts that are evenly spaced from behind the posterior end of the yolk sac to close to the tip of the tail. The distribution is not symmetrical between left and right sides; they are separated by 1 to 4 myomeres in dorsal view.

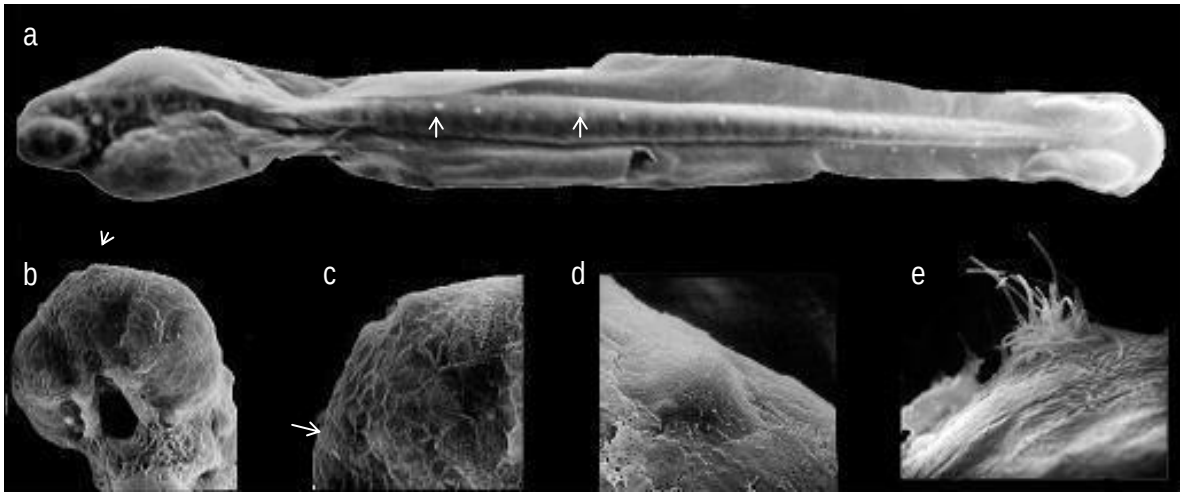

Figure 1. Yolk sac larva of *Engraulis ringens* (3.6 mm total length). a) Lateral view (30x) showing neuromasts along the trunk, b) head detail (280x) cephalic neuromasts, c) frontal area detail with olfactory channels (690x), d) trunk neuromast detail (1500x), e) cephalic region neuromast detail (5000x).

The neuromasts observed at hatching were the same size on the head and body in both species, indicating simultaneous formation during the embryonic period inside the egg; the cupulae grow in length as the larvae develop. The newer neuromasts form small cupulae that gradually increase in length as the larvae grow (Fig. 2). The cupulae of the first neuromasts from the head and trunk of newly hatched larvae measured 93 ± 28 and 121 ± 13 µm for anchoveta and 112 ± 52 and 138 ± 28 µm for common sardine without significant differences between species (t-test, $P = 0.161$).

Two days after hatching, the number of cephalic neuromasts increased with the addition of one pair above and one pair below the eye; these are the first of the supraorbital and infraorbital series, respectively. The number of neuromasts on the trunk does not increase during this period.

The numbers and position of neuromasts at hatching for anchoveta and common sardine larvae are similar to those observed in other Clupeiformes with comparable hatching sizes (and egg size). *Engraulis mordax* has 10-11 pairs (3-4 on the head and 6-7 on the trunk; Blaxter *et al*., 1983) and *Sardinops melanosticta* has 12 pairs (Matsuoka, 2001). Hatching larvae of *Clupea harengus* 8-10 (mm) have 6-8 remarkably larges pairs on the head and 10 pairs of neuromasts on the trunk (approximately 1 every four myomeres; O'Connell, 1981).

Initial hatching number and position of neuromasts are fairly constant within each of the two studied species. The results also show that there are no major

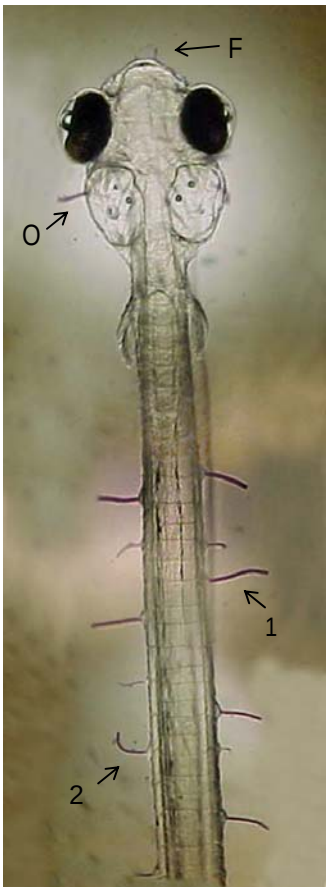

Figure 2. Late yolk sac larva of *Strangomera bentincki* (5.2 mm total length) stained with Janus Green. Dorsal view (40x) showing O: otic, F: frontal and trunk neuromasts already present at 1: hatching, 2: of later appearance.

variations between species as the intra-specific variation exceeds that between species. There seems to be a relative constancy in the number of the neuromasts formed before hatching and during the yolk sac period. The results recorded here fall within the range given for other distantly related groups such as *Micropogonias undulatus* (Perciformes: Scianidae; Poling & Fuiman, 1997) and *Gadus morhua* (Gadiformes: Gadidae; Blaxter, 1984), which develop 2-5 pairs on the head and 5-6 pairs on the trunk, respectively.

Yolk absorption at 12ºC occurs at 3.8 mm in anchoveta (4 days) and at 5.1 mm in common sardine (6 days). At this stage, the distribution and number of neuromasts is similar in both species, increasing on the average to 6 pairs on the head and 10-11 pairs on the trunk. On the head, the new neuromasts develop in the anterior portion of the supraorbital and infraorbital canals. On the trunk, the new neuromasts appear in middle intervening positions with respect to the initial ones; they also tend to form at different myomere level on both sides (Fig. 2). It has been hypothesized that this proliferation pattern in successive waves of intervening neuromasts confers greater early functionality (Fuiman *et al*., 2004).

Flexion larvae (9.1-10.6 mm) of both species have 17-18 pairs of trunk and tail neuromasts. Most of these are found along the mid side of the body, although a few occur slightly below that line. Additionally, 3 to 4 pairs of neuromasts begin to develop in the area of the developing hypural plates. The neuromasts on the head increase with the addition to the supraorbital and infraorbital series, and the appearance of the first on the preoperculomandibular series. However, the numbers begin to differ between species as anchoveta larvae show more neuromasts than those of common sardine at comparable stages.

In the largest post-flexion larvae analyzed (>11 mm), the trunk neuromasts increase to 30-39 pairs on both species. During this stage, a row of 4 neuromasts along the margin of hypural plates is noticeable (Fig. 3a). In other species, the neuromasts on the trunk continue to proliferate until they reach one per myomere; this is attained at 13-15 mm in *Engraulis mordax* (O'Connell, 1981). However, this had not occurred in either of the two studied species by day 25, when they had reached a maximum of 12 mm (*Engraulis ringens*) and 11.5 mm (*Strangomera bentincki*).

The cephalic neuromasts reach a maximum of 26 in anchoveta *vs* 34-36 in common sardine, respectively. There were no signs of cephalic canal formation in larval anchoveta (Fig. 3b). This process takes place at a larger size; it was recorded to begin after 15 mm in *Engraulis mordax* (O'Connell, 1981). The beginning of the invagination of an infraorbital neuromast was observed in 11.2-mm common sardine larvae (Fig. 3c).

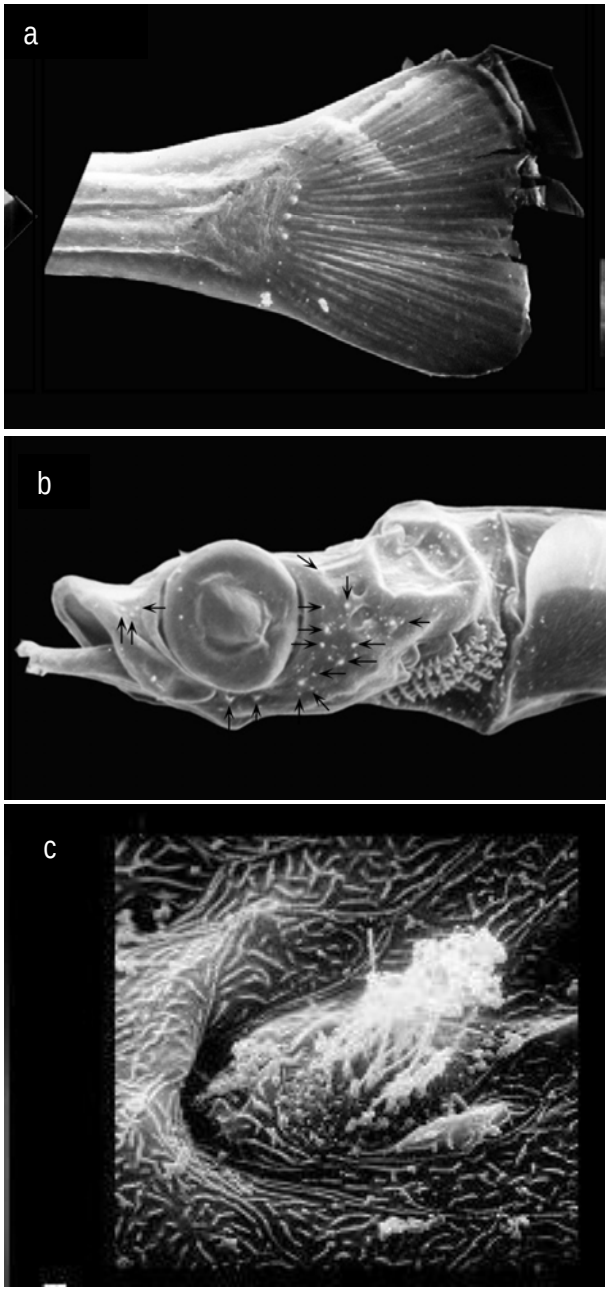

Figure 3. Details of neuromasts in post-flexion larvae. a) Detail of the tail (50x) of *Engraulis ringens*, b) lateral view of the head (50x) of *Engraulis ringens*, c) detail of the eye area (2500x) of a post-flexion larva of *Strangomera bentincki*.

When analyzing the rate of neuromast proliferation between species, no differences on the trunk were found (Fig. 4a, $F_{(1,58)} = 3.07$, $P = 0.084$). In the cephalic

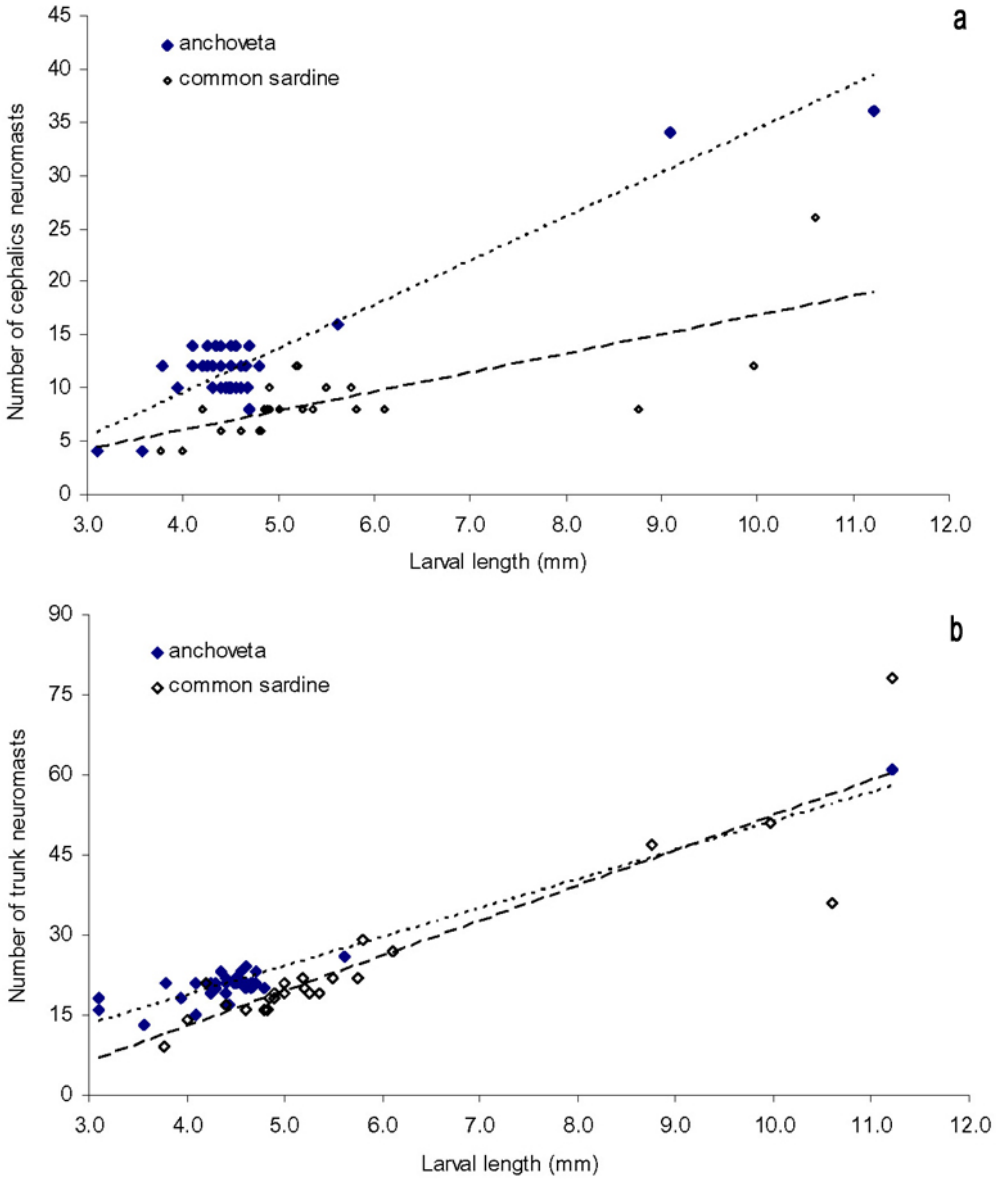

Figure 4. Relationship between larval length and number of neuromasts of *Engraulis ringens* (anchoveta) and *Strangomera bentincki* (common sardine) larvae. a) Neuromasts of the cephalic area, b) neuromasts of the trunk.

area, however, anchoveta larvae have a higher proliferation rate than those of common sardine (Fig. 4b, $F_{(1,58)} = 23.75$, $P < 0.001$). This was the only significant difference recorded between species.

Both species have the same number of hair cells per neuromast (12) at hatching (Figs. 1d-1e). This number is the same in cephalic and trunk neuromasts, shows little variation within or between species, and remains constant until after flexion. Hair cells begin to increase in number during post-flexion stages, as 15 were recorded in the largest larvae studied in both species. This pattern of initial appearance is similar to that of yolk sac larvae of *Thunnus orientalis* (Kawamura *et al*., 2003) but it differs from that of *Clupea harengus* larvae that hatch with 6-10 hair cells per neuromast. However, the latter species shows a major difference during early development as the eye becomes pigmented during egg development and vision seems to be a major factor of larval sensory perception.

The polarity of each cell was analyzed in those neuromasts in which each kinocilia and stereocilia could be clearly identified. In the two studied species, both head and trunk neuromasts had two polarities:

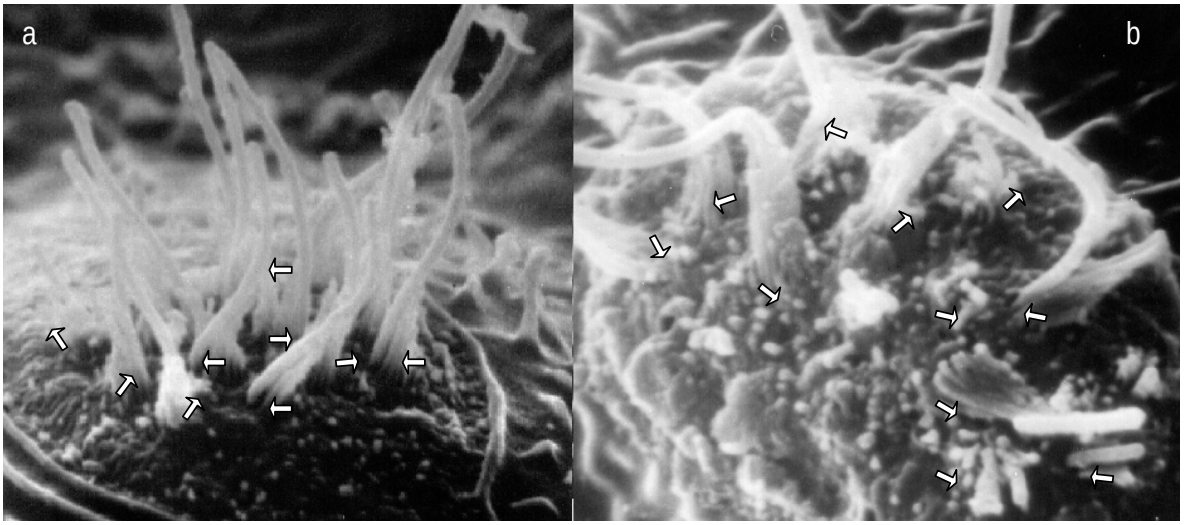

Figure 5. Detail of neuromasts from yolk sac larvae. a) *Engraulis ringens* (6000x), b) *Strangomera bentincki* (8000x). Each arrow indicates the polarity of the adjacent hair cell.

cranial-caudal and dorsal-ventral (Fig. 5). In other clupeiform, such as *Clupea* and *Alosa*, it is more common to find undirectional polarity, where neuromasts have either cranial-caudal or dorsal-ventral polarity (*e.g.*, Blaxter *et al.*, 1983; Shardo, 1996).

The structure of the lateral line of the adults shows a great deal of variation in Teleostei and it has been extensively used in phylogenetic analyses. However, the development pattern during the early ontogeny is less variable and seems to be determined by functional constraints (*e.g.*, Kawamura *et al*., 2003). Unrelated species with similar reproductive traits (*i.e.*, pelagic eggs and larvae) such as bluefin tuna (*Thunnus orientalis*, Kawamura *et al.*, 2003), anchoveta, and common sardine have early larvae that show similar neuromast number, distribution, number of hair cells, and polarity. Indeed, this can be even observed within Clupeiformes with different reproductive strategies and habitats; *Harengula jaguana* (demersal eggs), *Brevoortia tyrannus* and *Anchoa mitchilli* (pelagic eggs) (Higgs & Fuiman, 1998).

A more detailed analysis of closely related species with different early developmental features and environmental responses would help determine the relative importance of neuromast evolutionary history and function.

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