Taxonomic validation of *Encheliophis chardewalli* with description of calling abilities

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**Abstract**

*Encheliophis chardewalli* was described from a single cleared and stained specimen. Twelve years later, additional specimens were found in the lagoon of Moorea (French Polynesia) in association with their host, the sea cucumber *Actinopyga mauritiana*. These fish were used to consolidate the species diagnosis, to validate species status and to record sound production. This species is remarkable because of its ability to penetrate inside the cloaca of sea cucumbers having anal teeth and the fact this species is largely unknown despite it lives in lagoons in 1m depth. *Encheliophis chardewalli* produced three sound types: long regular calls made of trains of numerous pulses, short irregular calls characterized by a constant lowering of its pulse period and short regular call (or knock) made of 3 to 6 pulses. Comparison with other sympatric Carapini supports a large and distinct repertoire. Morphological characteristics could be the result of reduced body size allowing to penetrate inside a new host, thus avoiding competition and conflict with other larger sympatric Carapini species.

**KEYWORDS**

acoustic communication, Carapini, fish anatomy, pearlfish, sonic, *Actinopyga*

1 | **INTRODUCTION**

One remarkable association in the marine environment is between pearlfish (Carapidae) as symbionts with various marine invertebrates (holothuroids, asteroids, and bivalves) as hosts (Trott, 1970, 1981; Williams, 1984). Species of the genera *Onuxodon* and *Carapus* are commensals whereas *Encheliophis* species are parasites (Parmentier & Das, 2004). Members of this family produce sounds (Parmentier, Vandewalle, & Lagardère, 2003; Parmentier, Lagardère, Chancerelle, Dufrane, & Eckhaut, 2008; Kéver et al., 2014; Parmentier, Colleye, & Lecchini, 2016), which have been recorded (a) when several individuals of the same species were inside the same host (Parmentier, Vandewalle et al., 2003; Kéver et al., 2014), (b) when specimens of different species meet each other inside the host (Lagardère, Millot, & Parmentier, 2005), (c) when the fish were freely swimming (Parmentier, Fine, Vandewalle, Ducamp, & Lagardère, 2006) or (d) when competing for access to the host aperture (Parmentier, Fine et al., 2006). In the Carapini tribe grouping *Encheliophis* and *Carapus* (Markle & Olney, 1990; Parmentier, Castillo, Chardon, & Vandewalle, 2000), sound production is caused by contraction of two long primary sonic muscles (PSM) that run from the upper wall of the orbit to the anterior face of the swimbladder (Courteneay & McKittrick, 1970; Parmentier, Lagardère, Braquegnier, Vandewalle, & Fine, 2006). In some *Carapus* species (*C. boraborensis*, *C. mourlani*, *C. acus*), the PSM terminates in a complex tendon which forms a "hook" that fits over a tubercle on the anterior dorsal surface of the swim bladder (Parmentier et al., 2008). The sonic muscles contract slowly, pulling the anterior bladder rostrally. Sound is generated when extension trips the hook and causes the bladder to snap back to its resting position (Parmentier, Lagardère et al., 2006). *Carapus homei*, *Encheliophis gracilis* and *E. vermiops* lack the hook system and, consequently have direct insertion of their PSM onto the swim bladder indicating differences in the way sounds are produced. Interestingly, these acoustically related morphological characteristics correspond to important evolutionary steps within the Carapini. *Carapus homei* is the sister species of all *Encheliophis* spp, showing that parasite species have evolved from commensal species. *Carapus homei* already has the
sound-producing mechanism typically found in the parasite forms, but still has the head anatomy related to the commensal way of life, that is, the ability to feed hard elusive preys and not the host tissue (Parmentier, Lanterbecq, & Eeckhaut, 2016).

The genus Carapus contains seven species whereas five are found in Encheliophis. Within this genus, the description of Encheliophis char- dewalli was based on a single cleared and alizarin stained specimen deposited in the National Museum of Natural History in Washington (USNM 372738) (Parmentier, 2004). During field missions in French Polynesia, putative additional specimens have been found. In this study, we used them to confirm the species status of E. char dewalli. Moreover, we take advantage of these quite unusual living specimens to record their sounds and determine if they are distinct from sounds of other Carapini species.

2 | MATERIALS AND METHODS

Two E. char dewalli Parmentier 2004 (total length 6–10 cm) were collected in the lagoon of Rangiroa atoll (16°36′S, 143°42′W) in June 2008. They were euthanized with MS222 (Sigma-Aldrich, St. Louis, Missouri) and fixed in 5% paraformaldehyde. Eleven specimens (total length 6–10 cm) were found on the west side of bay mouth of Opunohu Bay (17°30′S, 149°50′W) at a depth from 1 to 3 m during May 2016. They were all found in the respiratory trees of the sea cucumber Actinopyga mauritiana. Fish caught in Moorea were stored with their host specimens in a community tank with running sea water. Sound recordings were made at 26°C in a glass tank (0.9 m × 0.5 m × 0.4 m). The recording protocol is the one usually used with carapids (Parmentier, Vandewalle et al., 2003; Parmentier, Fine et al., 2006; Parmentier et al., 2008; Kéver et al., 2014; Parmentier, Colleye et al., 2016). A specimen of the host was placed in the center of the tank, and several individuals were introduced successively into the aquarium. Sounds were recorded with a hydrophone placed above the sea cucumber (HTI Min-96, ~164.4 dB re 1V for a sound pressure of 1μPa; High-Tech Industries, Long Beach, MS) connected to a Tascam DR-05 recorder (44.1 kHz sampling rate; TEAC, Wiesbaden, Germany). The effects of reverberation, resonance, and tank size can induce artifacts on sounds recorded inside small glass tanks (Akamatsu, Okumura, Novarini, & Yan, 2002). The computed resonant frequency of the recording tank was 1.64 kHz; therefore a low-pass filter of 1.5 kHz was applied to all recordings. Sounds were digitized at 44.1 kHz (16-bit resolution), low-pass filtered at 1.5 kHz, and analyzed using AvisSoft-SAS Lab Pro 4.33 software. Only sounds with a good signal-to-noise ratio were used in the analysis. Temporal features were measured from oscillograms and frequency parameters were obtained from power spectra. The shape of the oscillogram indicates that the sounds were somewhat distorted, and pulse length may have been affected by tank acoustics (Parmentier, Tock, Falguére, & Beauchaud, 2014). The following sound parameters were measured: number of pulses in a sound; pulse period (measured as the average peak-to-peak interval between consecutive pulses, in ms), dominant frequency (Hz) and relative amplitude. The pulse periods of E. char dewalli sounds were compared with previously published results (Parmentier, Vandewalle et al., 2003; Lagardère et al., 2005; Parmentier et al., 2008; Parmentier, Colleye et al., 2016) using Kruskal-Wallis tests, followed by Dunn’s multiple comparison tests.

After recording, specimens were euthanized with MS222 (Sigma-Aldrich, St. Louis, Missouri) and fixed in 5% paraformaldehyde. Two specimens, one from each location (Rangiroa and Moorea) were cleared and stained with alizarin red S (Taylor & Van Dyke, 1985) to allow comparisons with the holotype (Parmentier, 2004). The fish were examined with a Wild M10 binocular microscope coupled with a camera lucida. Measurements were made to the nearest 0.01 mm. An additional specimen from Moorea was dissected to describe the anatomy of the sound-producing apparatus. Four complete specimens are now deposited in the Florida Museum of Natural History (catalog number UF 238789).

3 | RESULTS

3.1 | Diagnosis

As described for the holotype, specimens from Moorea and Rangiroa are mainly distinguishable from other Carapini species by the lack of pectoral and pelvic fins and seven branchiostegal rays. The comparison between the holotype and the specimens allows a correction, a clarification and the addition of meristic (Table 1) and morphometric (Table 2) features. Different anatomical features confirm the impression inferred from the holotype that this species could be paedomorphic (Parmentier, 2004). It exhibits arrested development because the pectoral fin, the upper jaws, the neural spines, the metapterygoid, the mesopterygoid, and the general body size are clearly not fully developed.

The body coloration has a number of remarkable features (Figure 1). Globally the body is translucent so that internal characters such as vertebral bodies, gills, and swimbladder are distinguishable. The main color pattern is beige with melanophores concentrated around the vertebral column and over myosepta. A higher melanophore concentration

| TABLE 1 | Meristic values in different Encheliophis char dewalli specimens |
|---------|------------------|------------------|------------------|
|         | USNM 372738 | Rangiroa | Moorea |
| Teeth on Dentary | 14 | 17 | 18 |
| Branchiostegal rays | 7 | 7 | 7 |
| Vertebrae | 107 | 97 | 106 |
| Precaudal vertebrae | 20 | 23 | 20 |
| Vertebrae to anal fin origin | 8 | 6 | |
| Vertebrae to dorsal fin origin | 20 | 17 | |
| Parapophysis origin | 4 | 4 | 4 |
| Vertebrae to vexillum origin | 4 | 4 | |
| Number of lateral line scales | Not visible | 59 | |
| Vertebrae to predorsal bone | 4 | 4 | |
causes a black tail tip. Rostrally it is possible to distinguish the white swimbladder although covered by many melanophores. However, the anterior tip of the swimbladder is laterally deprived of pigmentary cells providing two translucent areas that correspond to the position of the osseous swimbladder plate usually found at the level of the 3rd-5th vertebrae (5). In the pharyngeal region the lack of melanophores provides a lateral translucent area over the jaws and opercles and allows the clear distinction of heart and blood vessels, giving a reddish aspect to the head. Dorsally, it is possible to see the sagittal otoliths.

The sound-producing apparatus (Figure 2) is similar to those previously described in other Carapini species (Parmentier, Chardon, & Vandewalle, 2002; Parmentier, Gennotte, Focant, Goffinet, & Vandewalle, 2003; Parmentier, Vandewalle et al., 2003). The first two vertebrae display rodlike epineural that are movable in all directions, attach to each other and to the swim bladder by a common distal ligament. The third vertebra bears paired, broad, ossified plates that cover the anterior swimbladder and extend caudally to the 5th vertebra. The cylindrical swimbladder, extending from the 3rd vertebra to the 14th vertebra, is divided into three regions. The primary sound producing muscle is particularly long in this species: its origin is on the anterior upper wall of the orbit, at the level of the lateral ethmoid, and it inserts with a tendon directly on the anterior swimbladder without a hook (Parmentier et al., 2008; Parmentier, Lanterbecq et al., 2016). As usual in this taxa, the swimbladder fenestra, the second region, is situated just under the swim bladder plate and is thinner than surrounding regions. The third region comprised the remainder of the long and slender bladder. One pair of secondary sonic muscles joins the first epineurals with the epiotics.

3.2 Sounds

Fish do not make any obvious motions during sound production making it impossible to recognize the emitter or determine whether more than one fish was calling. Sounds were produced during encounters while circling the sea cucumber. Specimens have difficulty entering the host, and only 1 of 11 successfully entered. This observation suggests that the sea cucumber may have evolved a mechanism to hinder entry. The teeth around the cloacal opening appear to act as an impediment, and this echinoderm species does not house the other sympatric carapids that are all larger in diameter. Therefore all recorded sounds were produced when the fish was outside the host. In addition, no sound was emitted during the single penetration attempt.

Fish produced three kinds of sounds.

1. The long regular call (LRC/staccato) is made of trains of numerous pulses (range from 15 to >80) with a uniform pulse period of \((235 \pm 21)\) ms \((\text{mean} \pm \text{SD})\) and a pulse duration of 12–20 ms (Figure 3). The power spectrum has two main frequencies: 95 Hz and around 330 Hz. Dominant frequency is related to sound level, and higher amplitude sounds have frequencies around 330 Hz.

2. The second sound type is a short irregular call (SIC) similar to a drum with a decreasing pulse period (Figure 4). In calls having five

| TABLE 2 | Morphometric characters in different Encheliophis chardewalli specimens |
| --- | --- | --- | --- | --- | --- |
| Rangiroa | 7.16 | 3.94 | 2.92 | 1.7 | 86 |
| Moorea | 10.42 | 5.1 | 2.86 | 1.88 | 104.5 |
| | 6.62 | 2.96 | 1.80 | 1.10 | 61.5 |
| | 8.36 | 4.02 | 2.62 | 1.70 | 90 |
| | 8.46 | 3.98 | 2.60 | 1.6 | 83.7 |

Values are in mm.
pulses or more, the last pulse period is usually half the duration than the first period. These sounds are made of 3 to 8 pulses ($5 \pm 1$, $n = 59$) with a duration of $181 \pm 50$ ms ($n = 185$). The main pulse period is $25 \pm 7$ ms ($n = 234$) but oscillates between 42 and 12 ms. The dominant frequency of the call is $394 \pm 112$ Hz ($n = 58$) which is close to the dominant frequency of each pulse ($390 \pm 110$, $n = 230$).

3. The last sound (Figure 4) is a short regular call (SRC/knock) made of 3 to 6 pulses ($4 \pm 0.8$, $n = 42$) having a constant pulse period of about $53 \pm 6$ ms ($n = 128$). The call duration ranges from 77 to 260 ms ($174 \pm 44$, $n = 43$) and the dominant frequency is $407 \pm 102$ Hz ($n = 147$).

In most cases (84%), SRCs are followed by SICs (Figure 4). There is usually $84 \pm 56$ ms ($n = 90$) between the end of the SRC and the beginning of the SIC. The amplitude of SRC is ca. 60% lower than drums (t-student, $t = 21.8$, d.f. = 65, $p < .001$). Because *E. chardewalli* does not move during sound production, it is not possible to claim if sounds were made by two different specimens or not.

The comparison of the shapes of each pulse from the different kinds of sound seems to indicate that pulses from staccatos and SICs are constructed in the same way and most probably result from myo-electrical activity of the sound-producing organ.

**FIGURE 2** Left lateral view of the anterior part of the sound-producing system in *Encheliophis chardewalli*. Yellow dotted lines correspond to the position of the swimbladder fenestra.

**FIGURE 3** Long regular calls produced by *Encheliophis chardewalli*. Oscillogram (a) and enlargement of some pulses (b). Oscillogram and corresponding spectrogram (c). Spectral analysis information: sampling frequency 44 100Hz; Windows: Flat Top (filter bandwidth: 15Hz); FFT length: 1024.

**FIGURE 4** Different kinds of sounds in *Encheliophis chardewalli*. Oscillogram (a) and enlargement of some pulses (b) with SRCs or knocks (1), and SIC (2). Oscillogram and corresponding spectrogram of both kinds of sounds (c). Spectral analysis information: sampling frequency 44 100Hz; Windows: Flat Top; FFT length: 1024.
from the same mechanism (Figure 5). In this case, it seems that the
difference between both kinds of sound would be simply related to
differences in the rate emission. On the other hand, the shape of
the knock pulse appears to be different and would result from a dif-
ferent way of sound producing.

4 | DISCUSSION

The new specimens collected at Moorea show a series of key charac-
ters that corresponds undoubtedly to *E. chardewalli*, including the lack
of pectoral fins, seven branchiostegal rays and short premaxilla (Par-
mentier, 2004). We designate specimens from this study as metatypes
that validate the species’ status. However, we adjust the original
description: some specimens possess reduced pelvic bones, a single
row of scales at the lateral line, upper jaws reaching the coronoid pro-
cess of the mandible, up to 18 conical teeth on the mandible and a pre-
dorsal bone at the level of the 4th vertebra (Table 1). An additional
distinctive character is the number of vertebrae anterior to the origin
of the dorsal fin, between 17 and 20, which is higher than in other
carapid species (but *E. vermicularis* has 16–19 vertebrae) (Markle &
Olney, 1990). Morphological features suggest that *E. chardewalli* is a
paedomorph with arrested development. The life cycle of Carapidae is
divided into four stages: the vexillifer and tenuis larvae, juveniles and
adults (Olney & Markle, 1979). The vexillifer larva provides the pelagic
dispersal stage. Later, the tenuis larva increases in length substantially
before a deep metamorphosis (within the host) that includes a shorten-
ing of the body (Parmentier, Lecchini, & Vandewalle, 2004) before the sexually
mature adults. The anatomy and the small body size associated with
the straight body diameter suggest the adults still have the morphology
of the tenuis (Parmentier, Lanterbecq et al., 2016).

*Encheliophis chardewalli* was found in the surf redfish *A. mauritiana*,
a sea cucumber that usually dwells in shallow waters from the surface
to 20m depth. It is quite surprising to find carapid fish inside this sea
cucumber because it possesses anal teeth around its cloacal opening
(Moss & Murchison, 1966). These teeth complicate penetration by
restricting the anal aperture and could harm the fish. The narrow
guarded opening probably explains the particularly narrow dimensions
of *E. chardewalli* (Table 2) and the absence of pectoral fins. However, *E.
chardewalli* was already supposed to be paedomorphic given that its
skeletal morphology has a series of features (narrow sizes, poor devel-
opment of the vertebra, lack of pectoral fins, tenuis aspect) that appa-
rently represent arrested development (Parmentier, 2004). This general
morphology could be the result of a reduction of body size allowing to

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TABLE 3 Synthesis of the different sounds known in Carapini species

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<thead>
<tr>
<th>Species</th>
<th>Staccato</th>
<th>Knock</th>
<th>Double-pulse</th>
<th>Hum</th>
<th>SIC</th>
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<tr>
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<td><em>Carapus mourlani</em></td>
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<td><em>Carapus acus</em></td>
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FIGURE 5 Typical shapes of pulses belonging to the different kinds of sounds in *Encheliophis chardewalli*. The double-head arrow shows
the pulse duration. The ending part that looks like a sine wave is a tank reflection

FIGURE 6 Comparison of the pulse period (mean and S.E.)
between knocks and staccatos of different Carapini species. All the
sounds are significantly different
penetrate inside new host, avoiding competition and conflict with other sympatric Carapini species (C. boraborensis, C. mourlani, C. homei, E. gracilis).

In E. chardewalli, the comparison of the shapes of each pulse from the different kinds of sound seems to indicate that pulses from staccatos and SCIs are constructed in the same way and most probably result from the same mechanism (Figure 5). In this case, it seems that the difference between both kinds of sound would be simply related to differences in the rate emission. On the other hand, the shape of the knock pulse appears to be different and would result from a different way of sound producing. The use of secondary sonic muscles could be involved because the contraction of these muscles necessarily implies the forward and outward displacement of the posterior tips of the epi-neurals, and consequently of the anterior part of the swimbladder (Courtenay & McKittrick, 1970; Parmentier, Gennotte et al., 2003).

Sound production ability is well developed in the Carapini with recorded species producing different sound types. Pulses can be produced as a single unit or in long regular trains (staccato) in C. boraborensis (Parmentier, Vandewalle et al., 2003). They occur in groups of 2–6 pulses, suites of double-pulses, staccatos and hums in C. mourlani (Parmentier, Fine et al., 2006; Parmentier, Colleye et al., 2016). They can be single, suites of double-pulses, or in groups of three pulses to seven pulses in C. homei (Parmentier, Vandewalle et al., 2003; Lagardère et al., 2005). They are single or in groups of five to six pulses in E. gracilis (Parmentier, Vandewalle et al., 2003; Parmentier et al., 2008). Only single pulses were recorded in C. acus (Parmentier, Fine et al., 2006), suggesting this species most probably produces additional kinds of sounds. Single-pulsed sounds and long trains are emitted by O. fowleri (Kéver et al., 2014). Encheliophis chardewalli produces at least three different sound types. Unfortunately, it is currently not possible to associate these sounds with a specific message because they are produced during similar conditions. We note that bird species often produce different calls in the same setting (Brown, 1975). It also means the complete repertoire of each recorded species was unlikely present. Nevertheless, based on current knowledge, E. chardewalli is the only Carapini species able to produce the SCI since single pulses, staccatos, and knocks can be produced by other species (Table 3).

Recordings in tanks can distort the signal in different ways. Comparative experiments on the red drum, Sciaenops ocellatus indicate, however, that potential distortion does not affect the pulse period, or the number of pulses or the period between consecutive calls (Parmentier et al., 2014). The comparison of staccatos and knocks indicated the pulse periods of each species (Figure 6) are significantly different (Kruskall-Wallis, p<0.001) indicating each species can be distinguishable. It can be helpful in future field studies since all these species were found in the bay of Opunohu (Moorea) and for some of them in the same host species (Parmentier & Vandewalle, 2005).

During our laboratory observations, it was not possible to associate the sounds with a given behavior or to identify the emitter since no associated movements accompanied sound production. The high diversity of sounds in all studied species clearly supports the importance of this behavior in these nocturnal symbiotic species. It is also likely that sound production exits in all carapids since all species possess sound producing muscles associated with the swimbladder (Parmentier et al., 2002).

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REFERENCES


(Eds.), Functional and ecological vertebrate morphology (pp. 139–151). Maastricht: Shaker Publishing.

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