Sound production mechanism in the semiterrestrial crab Neohelice granulata (Brachyura, Varunidae)^{a)}

María P. Sal Moyano,^{1,b)} María Ceraulo,² Salvatore Mazzola,² Giuseppa Buscaino,² and María A. Gavio¹

¹Instituto de Investigaciones Marinas y Costeras (IIMyC), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)–Universidad Nacional de Mar del Plata (UNMdP). Funes 3350, Mar del Plata, 7600, Argentina

²Institute of Anthropic Impact and Sustainability in marine Environment (IAS)–CNR National Research Council Via del Mare 3 91021 Torretta Granitola, Italy

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Very few studies of sound production in the Brachyura have simultaneously identified the type of individuals (e.g., sex) producing acoustic signals, the structures involved in making sound and the social context. The emission and type of sound signals in *Neohelice granulata* were previously characterized, but the sex and the body structures involved in the sound production mechanism were not determined. In the present study, experiments conducted in the laboratory demonstrated that acoustic signals were produced by males through an up–down movement of the cheliped by rubbing the merus against the pterygostomial area of the carapace. The micromorphology of the merus showed that it has a ridge of tubercles which may act as a plectrum, while the pterygostomial area bears tubercles and might function as the pars stridens. Acoustic signals were displayed more frequently in the presence of receptive females. The authors propose that *Neohelice granulata* males use their chelipeds to produce sound signals in a mating context, probably to attract the receptive female and/or to repel other males when a receptive female is present. Thus, the display might have a reproductive function influencing mate choice. © 2019 Acoustical Society of America. https://doi.org/10.1121/1.5128473

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I. INTRODUCTION

Studies on sound production mechanisms in crabs, either terrestrial, semiterrestrial, or aquatic, have been widely reported (e.g., Salmon, 1965; Horch, 1971; Abele *et al.*, 1973; Field *et al.*, 1987; Boon *et al.*, 2009; Buscaino *et al.*, 2015). However, most of these studies were focused on ocypodid crabs in which the sounds were transmitted either as substrate vibrations or as airborne stimuli (e.g., Salmon, 1967; Horch and Salmon, 1969; Salmon and Horch, 1972; Horch, 1975, Budelmann, 1992; Clayton, 2012), while there have been few studies of species using under water (aquatic) acoustic communication.

Different mechanisms of sound production have been described in crabs, including striking of a body part on the substrate, vibration of appendages, respiration, and rasping involving the cephalothorax and/or the appendages (Guinot-Dumortier and Dumortier, 1960; Salmon and Horch, 1972). The latter mechanism, using the friction of appendages against the cephalothorax, is considered the primary mode of stridulation, employing a scraper or "plectrum" bearing a tapering edge or denticulations which is moved against a file or the "pars stridens" composed of tubercles or ridges (e.g., Dumortier, 1963; Clayton, 2005). This type of mechanism of

sound production is described in many species of decapods such as spiny lobsters (Patek, 2001; Patek *et al.*, 2009), hermit crabs (Field *et al.*, 1987), terrestrial crabs (Abele *et al.*, 1973), and mangrove crabs (Boon *et al.*, 2009). However, it can stand out that stridulation may also occur without a specific stridulatory structure or use of an appendage. For example, in terrestrial hermit crabs a "chirping" sound is produced by the rubbing of the abdomen tip against the shell apex (Imafuku and Ikeda, 1990).

Studies on acoustic communication in brachyuran crabs have been related mostly to sexual behavior (generally mate attraction, e.g., Salmon, 1967; Popper *et al.*, 2001; Parker *et al.*, 1998; Buscaino *et al.*, 2015) and the defense of territories (by displaying agonistic interactions, e.g., Seiple and Salmon, 1982; Boon *et al.*, 2009). However, few studies have been conducted to jointly identify the type of individuals producing sounds (e.g., male or female), the structures involved in sound production and the social context.

The semiterrestrial burrowing crab *Neohelice granulata* is a key species inhabiting the intertidal zone of estuaries, salt marshes, and mangroves of the south-western Atlantic Ocean. It is considered an emergent animal model since during the last 30 years there was an explosion of publications in international journals dealing with its ecology, physiology, toxicology, and behavior (Spivak, 2010). However, there is a lack of studies on acoustic communication in this species. The fact that it is a semiterrestrial crab may indicate

^{a)}This paper is part of a special issue on the effects of noise on aquatic life. ^{b)}Electronic mail: salmoyan@mdp.edu.ar

that this species potentially uses acoustic signals involved in intraspecific acoustic communication as described in other semiterrestrial crab species of the Grapsoidea (e.g., Abele *et al.*, 1973; Boon *et al.*, 2009) and Ocypodoidea (e.g., Horch, 1975; Clayton, 2012). Additionally, *N. granulata* belongs to the Varunidae family where several species have been described to have specific and conspicuous stridulating structures that were proposed to be used in acoustic communication (Guinot *et al.*, 2018).

The study of N. granulata mating system and the reproductive strategies displayed has been widely assessed (Sal Moyano et al., 2012a; Sal Moyano et al., 2012b; Sal Moyano et al., 2014a; Sal Moyano et al., 2016a; Sal Moyano et al., 2016b). Crabs live in burrows constructed in the mud flat or saltmarsh and reach high densities, up to 60 burrows m⁻² (Iribarne et al., 1997). Receptive females abandon their burrows and search for male burrows, initiating a courtship behavior which consists of "sensing" males inside their burrows (Sal Moyano et al., 2014b). Mating was observed inside male burrows or on the surface when females are intercepted by males (Sal Moyano et al., 2012a). Mating (inside or outside burrows) occurs during low or high tide: during low tide water recedes and burrows become totally exposed while during high tide burrows are totally submerged (Sal Moyano et al., 2014a). The use of chemical signals in courtship and mating has been demonstrated to occur in this species, while visual signals were shown to not be involved (Sal Moyano et al., 2014a). The aquatic sound emission and the type of sound signal produced was previously characterized in this species and associated with a reproductive context (Filiciotto et al., 2018; Filiciotto et al., 2019). However, no studies were conducted to recognize which sex and what structures were involved in sound production. Thus, our aim was to identify the sound production mechanism, the sex of the individual producing the sound signals, and an aquatic context in which this behavior occurs.

II. MATERIAL AND METHODS

A. Collection and maintenance of crabs

Mature crabs (CW > 19 mm, López Greco and Rodríguez, 1998) were collected (N = 80) during the reproductive season from November 2017 to February 2018 at Mar Chiquita Coastal Lagoon (37°45'S, 57°19'W, Buenos Aires, Argentina), and transported to the laboratory. Carapace width (CW) was measured with calipers at a precision of ± 0.1 mm. Females collected ranged from 24 to 28 mm CW (N = 40), while males ranged from 26 to 32 mm CW (N = 40). Males and females were held separately under laboratory conditions in glass aquaria $(30 \times 35 \times 25 \text{ cm})$ containing filtered seawater (3 L). A maximum of four crabs of similar size per aquaria was allocated avoiding effects of density and size on behavior (Sal Moyano et al., 2016b). Acclimation conditions were light cycle 14 L/10D, temperature 24 ± 3 °C, salinity 23 ppt, and continuous aeration. Individuals were fed daily with rabbit pellet food and water was changed after feeding. After 48 h crabs were considered acclimated and ready to use in experiments. Individuals were held for a maximum of one month under the laboratory conditions, after this, they were replaced by new crabs collected from field.

Females were monitored daily to detect receptivity by checking their vulvae opercula under a stereomicroscope. When the vulvae operculum became mobile, it could be pushed inwards with fine forceps, the female was considered receptive and immediately used in the experiments (Sal Moyano *et al.*, 2012b).

B. Experimental setup

The experimental PVC tank was circular (1.2 m diameter and 1.5 m depth), covered internally with black rubber to enhance crab's visualization and to avoid incidental sound and substrate-borne vibrations produced by crab locomotion within the tank. The tank was filled with filtered seawater (1.2 m depth). A calibrated hydrophone (model Reson TC4013, with a sensitivity response of $-211 \pm 3 \,\mathrm{dB}$ re $1 \,\mathrm{V}/\mu\mathrm{Pa}$ between a wide frequency range of 1 Hz and 150 kHz,) was located in the center of the tank at 20 cm depth. It was coupled with a preamplifier (1-MHz bandwidth single-ended voltage and a high-pass filter set at 10 Hz, 20 dB gain, Avisoft Bioacoustics), connected to a digital acquisition card (Avisoft UltraSoundGate 116h) managed by the Avisoft Recorder USGH software (Avisoft Bioacoustics). The sampling frequency of the hydrophone was set at 300 000 sample per second. A GoPro camera (set at 30 frames per second) was positioned centrally to monitor the tank bottom.

Two experimental trials were performed (1) a receptive female + two males, and (2) a non-receptive female + two males. Nine replicates per trial were conducted (N individuals = 54). Different individuals (both males and the female) were used in each replicate. Because size in this species is a phenotypical character determining dominance and the establishment of social hierarchies (Sal Moyano et al., 2016b), the size difference between males was never larger than 1 mm. In all trials, a maximum size difference of 4 mm among males and females was allowed following Sal Moyano et al. (2014b). Crabs (both males and the female at the same time) were placed in the center of the tank with a net. Individuals were left for 5 min to acclimate to their new surroundings, whereupon the experiment began and finished 1 h later. When the experiment began, both the hydrophone and GoPro camera were started synchronously, avoiding any delay in seconds. No shelters or structures were available because previous laboratory studies conducted with this species demonstrated that mating can occur in the absence of refuges.

C. Video and acoustic analysis

The video and the acoustic signal emissions were analyzed simultaneously. Visual inspection of the video and identification of the acoustic signals using the Avisoft-SASLab Pro software (Avisoft Bioacoustics) was conducted by an operator. An example of the pattern of the acoustic signals we found during the experiment is shown in Fig. 1 (for more acoustic details see Filiciotto *et al.*, 2019). Each rasp was counted as an acoustic signal. Repeated rasps were



FIG. 1. (Color online) Oscillogram (top) and spectrogram (middle) of a rasp train (FFT length of 1024, with a Hamming window, time segments overlap 75%, x axis: time; y axis: frequency on a linear scale; sampling frequency 300 kHz). Below: power spectrum density (dB re 1 μ Pa2/Hz) of a selected rasp (rectangle) (above line) and tank background noise (below line).

considered as a "rasp train." Once the acoustic signals were recognized, they were matched with the movement involved in producing them. The number of acoustic signals and movements that produced them were quantified. A display was considered to occur each time an individual began and ended doing the movements that produced the acoustic signals. The analyses of the video images allowed us to identify the individual making the sound. The individual (both males named as: male a, male b, or the female) performing a display was visually identified. Both males were not previously differentiated by marking because visual inspection of videos images clearly enabled us to differentiate them all during the trial.

Analyses of the video images also involved the quantification of the interactions among males. An interaction occurred each time both males contacted each other with their chelipeds (without considering the time duration of the interaction). The acoustic signals produced by the cheliped



FIG. 2. (Color online) *Neohelice granulata* male showing the row of setae (white arrows) and the ridge of tubercles (black arrow) of the cheliped merus internal side and the pterygostomial area (circle).

contacts were not considered further given that they were the result of the physical interaction between chelipeds.

D. Morphometric analysis

The body structures involved in the sound production mechanism were identified: the pterygostomial area and both cheliped meri (Fig. 2). Mature males and females from the laboratory (N = 5 for each sex) were sacrificed by placing them in a freezer at -20 °C for approximately 30 min. The body structures were dissected, dried at room temperature (23–25 °C), and coated with gold–palladium, to be inspected under a Jeol JSM-6460LV scanning electron microscope. The micro-morphology of the structures was described and characterized.

E. Statistical analyses

To test for differences in "number of acoustic signals," "number of displays," and "number of movements" between trial 1 (two males and a receptive female) and trial 2 (two males and a non-receptive female) a Mann-Whitney test was performed. The number of movements and displays by the two males in both trials were compared. The number of cheliped male interactions were similarly compared between both trials. A correlation analyses between the number of movements and the number of acoustic signals, considering jointly the data of both trials, was conducted.

III. RESULTS

A. Video and acoustic analysis

The movements that produced the sound signals involved an up-down cheliped movement (either right or left cheliped), rubbing its merus against the pterygostomial area. The number of acoustic signals, up-down cheliped movements and displays were higher in trial (1, receptive female) compared to trial (2, non-receptive female) (Fig. 3; Mann-Whitney tests: Z = 2.2, p < 0.05; Z = 2.43, p < 0.05, Z = 2.38, p < 0.05). In both trials, there were differences in the number of displays performed by the individuals: only males engaged in displays and they were made mostly by one of the two males [Fig. 4(a); Mann-Whitney tests: trial 1, Z = 2.39, p < 0.05; trial 2, Z = 2.52, p < 0.05]. The number of cheliped movements did not differ among individuals in both trials: the number of movements per display was the same among males [Fig. 4(b); Mann-Whitney tests: trial 1, Z = 1.8, p = 0.07; trial 2, Z = 1.23, p = 0.21].

Interactions among males using their chelipeds occurred mostly in the presence of a receptive female: the number of cheliped interactions was higher in trial (1) compared to trial (2) (Mann-Whitney: Z = 1.99, p < 0.05). The number of up–down cheliped movements was linearly related to the number of acoustic signals (Fig. 5; $R^2 = 0.75$, F = 92.2, p < 0.001). No observations of males trying to capture the female or guarding her were visualized in any trial.

B. Morphometric analysis

The analyses of the micro-structures of the pterygostomial area showed the presence of a suborbital ridge and two differentiated areas, above and below it [Fig. 6(a)]. The suborbital ridge was characterized by the presence of tubercles and long setae (four times longer than tubercles) with long pinnules located all along their length, and infracuticular insertion [Fig. 6(b)]. The area above the suborbital ridge was characterized by an alternation of tubercles and short setae (similar size as the tubercles) whose tips bear grouped short pinnules resembling a "duster" [Figs. 6(c) and 6(d)]. The area below the suborbital ridge was characterized by an alternation of tubercles and a medium seta (twice the size of the tubercles) type characterized by the presence of long



FIG. 3. Results of the Mann-Whitney tests showing differences in the (a) number of acoustic signals, (b) number of displays and, (c) number of up-down cheliped movements; between both trials. Trial 1: receptive female + male + male (N replicates = 9), trial 2: non-receptive female + male + male (N replicates = 9). * Significant differences (p < 0.05).



FIG. 4. Results of the Mann-Whitney tests showing (a) differences in the number of displays performed by male a (Ma), male b (Mb) or the female (receptive and non-receptive, RF and NRF, respectively), between trial 1 (receptive female + male + male) and trial 2 (non-receptive female + male): only males engaged in displays and they were made mostly by one of the two males; (b) differences in the number of up–down movements performed by male a (Ma), male b (Mb) or the female (receptive and non-receptive, RF and NRF, respectively), between trial 1 (receptive female + male) and trial 2 (non-receptive female + male) and trial 2 (non-receptive female + male) and trial 2 (non-receptive female + male): no differences between trials were found. * Significant differences (p < 0.05).

pinnules at the base, a tip lacking pinnules and infracuticular insertion [Figs. 6(e) and 6(f)].

The image analyses of the micromorphology of the male cheliped merus showed the presence of a setae type which were grouped forming a fringe on its internal median side and a ridge of rounded tubercles in its inferior region [Fig. 7(a)]. The detailed view of the setae showed that they had a serrate form on one side of the tip due to the presence of conical structures which resemble "teeth," and infracuticular insertion [Fig. 7(b)]. The internal side of the cheliped merus of females showed the same grouped serrate setae forming a fringe in its median region and the ridge of tubercles in its inferior area, although tubercles had a conical and less pronounced form [Fig. 7(c)].

The detailed analysis of the merus up-and-down movements in the video images may indicate that sound is produced when the ridge of tubercles on the inferior region of the merus is rubbed against the tubercles of the area below



FIG. 5. Linear correlation between the number of up-down cheliped movements and the number of acoustic signals performed by males in both trials.

the suborbital ridge. In this way the merus may be acting as a plectrum and the area below the suborbital ridge as the pars stridens. The meral movements were observed all along the pterygostomial area below the suborbital ridge. Neither the area above the suborbital ridge nor the suborbital ridge itself seems to be involved in the sound production by the merus movement. The presence of setae in alternating with the tubercles in the pterygostomial area do not seem to have a role in the sound production mechanism. Similarly, the serrate setae of the internal median region of the merus seem to do not have enough stiffness to act as the plectrum and rub against the tubercles of the pterygostomial area.

In both males and females, the same micromorphology of the structures described for the pterygostomial area was observed. However, differences in the form of tubercles of the merus ridge were found: males showed prominent rounded tubercles while females presented less pronounced tubercles and with a conical form.

IV. DISCUSSION

Here, we found that *N. granulata* males may produce acoustic signals related to an up–down movement of the cheliped merus, by rubbing the tubercles against the tubercles of the area below the suborbital edge of the pterygostomial area. This would occur in a reproductive social context because merus movements and their respective acoustic signals were registered more frequently in the presence of a receptive female.

The acoustic signals found here are similar to the ones characterized previously in *N. granulata* by Filiciotto *et al.* (2018); Filiciotto *et al.* (2019). Such a signal was defined as a large band multi-pulse rasp characterized by a train with a highly variable number of impulses, similar to the emission pattern of other rasps produced by stridulating crustaceans (Patek, 2001; Buscaino *et al.*, 2011a; Buscaino *et al.*, 2011b). For example, in two species of sesarmid crabs



FIG. 6. *Neohelice granulata* males: microstructures of the pterygostomial area. (a) General view, the arrow indicates the suborbital ridge that delimits the upper area above (aa) and the lower area below (ab) it. (b) Detailed view of the suborbital ridge with the tubercles (arrow) and the long pinnate setae (S), and the areas above (aa) and below (ab) it. (c) General view of the upper area showing the alternation of tubercles and short pinnate setae. (d) Detailed view of the upper area showing a tubercle and a short pinnate seta. (e) General view of the lower area showing the alternation of tubercles and setae. (f) Detailed view of a tubercle and a base-pinnate seta.



FIG. 7. *Neohelice granulata*: microstructures of the cheliped merus. (a) General view of the internal side of a male merus showing the grouped setae (S) forming a fringe in its median region and a ridge of tubercles in its inferior area (arrows). Inset: detailed view of the prominent rounded tubercles. (b) Grouped setae showing the serrate form (black arrow) on one side of its tip, and the infracuticular insertion (white arrow). Inset: detailed view of a serrate setae showing the conical teeth. (c) General view of the internal side of a female merus showing the grouped serrate setae (S) forming a fringe in its median region and a ridge of weak conical tubercles forming its inferior area (white arrow). Inset: detailed view of the conical and less evident tubercles.

(*Perisesarma* spp.), the presence of multiple sound pulses composed of a rasp train using the same stridulation movements were reported (Boon *et al.*, 2009). These sound signals were produced only by males during agonistic interactions while fighting for a territory, with only one male displaying at a time (Boon *et al.*, 2009). Similarly, in *N. granulata*, only males displayed acoustically and one male at a time. Besides, there were more male cheliped contact interactions in trials with a receptive female. Thus, it is proposed that *N. granulata* sound display is related to an agonistic behavior among males, as occurs in sesarmid crabs (Boon *et al.*, 2009), but associated with a reproductive context.

The display of agonistic interactions among males for burrow territory was previously found in this species (Sal Moyano et al., 2012a). Burrows are resources used for mating because males use them to attract receptive females (Sal Moyano et al., 2012a). Considering this scenario, we propose that N. granulata males use the acoustic signal described here to attract receptive females or to repel other males when a receptive female is available. The hypothesis might be reinforced given that it has been demonstrated that N. granulata use chemical but not visual signals (during low and hide tide, thus, when exposed or covered with water) in a reproductive context because males are not visible inside burrows when receptive females look for them (Sal Moyano et al., 2014). Thus, the use of acoustic signals may constitute an additional important complement in signaling the reproductive behavior of this species. Similarly, the use of acoustic signals in a sexual scenario was previously described in fiddler crabs (Salmon and Atsaides, 1968; Popper et al., 2001; Takeshita and Murai, 2016; Mowles et al., 2017) and two species of Ovalipes, O. catharus and O. trimaculatus (Parker et al., 1998; McLay, 1988; Buscaino et al., 2015). The absence of a courtship or mating behavior in any trial of the present study could be related to the short experimental time (1 h). However, we considered that it was enough time to allow recognition and communication among individuals according to previous studies about mating behavior conducted in this species (e.g., Sal Moyano et al., 2012a; Sal Moyano et al., 2014a, Filiciotto et al., 2019).

Although *N. granulata* does not appear to actively contact the sediment when producing sound, the locomotory appendages/body are in contact with the substrate during sound production which provides a potential transmission channel. Thus, even though we did not measure seismic detection in the present study, substrate-borne vibration reception could constitute an alternative form of communication in this species, as it was demonstrated to occurred in other semiterrestrial and aquatic crustaceans and invertebrates (e.g., Salmon and Horch, 1973; Taylor and Patek, 2009; Roberts *et al.*, 2016).

The mode of stridulation by using the friction of appendages against the carapace, with one acting as a plectrum and the other as a pars stridens was first defined by Guinot-Dumortier and Dumortier (1960). This type of stridulating system was reported in several species of Varunidae such as *Leptograpsodes octodentatus* (as *Brachynotus*), *Cyclograpsus audouinii*, *Hemigrapsus sexdentatus*, *Metaplax crenulata* (as *Hemigrapsus crenulatus*) and *Thalassograpsus harpax* which exhibit stridulatory structures in both sexes, with the pars stridens formed by the suborbital margin and the plectrum located on the inner margin of the cheliped merus (Davie and Ng, 2007; Guinot *et al.*, 2018). These conspicuous stridulatory structures of varunids are so specialized that an acoustic function could not be doubted (Guinot *et al.*, 2018). Similarly, in the genus *Helice*, the stridulating system is composed of a suborbital crest with tubercles—pars stridens—and a horny crest in the upper face of the cheliped merus—plectrum—(Schmitt, 1965; Guinot-Dumortier and Dumortier, 1960; Sakai *et al.*, 2006). However, no studies were conducted on sound production and stridulating in those crabs.

In the varunid *N. granulata*, the tubercles of the inferior inner margin of the cheliped merus resemble the plectrum, while the tubercles of the area below the suborbital ridge seem to be the par stridens. Sakai et al. (2006) suggested that in the genera Austrohelice and Neohelice (Varunidae) there is no plectrum and the suborbital ridges are isomorphic in both sexes, proposing that it is the least advanced condition and that these two genera do not stridulate. Furthermore, in the specific case of Neohelice granulata, Sakai et al. (2006) proposed that this species do not have a stridulatory mechanism because the suborbital crest bears two unequal rows of isomorphic granules in both sexes but the cheliped merus lacks the typical crest in the plectrum of *Helice tridens.* However, in the present study, the prominent tubercles forming a ridge in the inferior inner surface of the cheliped merus seem to be the plectrum. Our results show the emission of acoustic signals demonstrate that this constitutes a stridulating system in N. granulata.

The presence of a similar stridulating system producing sound signals has been described in some other species of crabs. In the terrestrial crab *Gecarcinus quadratus* (Eriphiidae), the merus and pterygostomial area bear tubercles and the friction between them produce the aerial sound, presumably involved in an anti-predatory display (Abele *et al.*, 1973). In the case of two species of *Perisesarma*, the stridulatory structures involved tubercles on the chelae dactylus that engage in a friction movement against a double serrate setae row on the propodus when one claw remains stationary while the rasping claw is moved up and down (Boon *et al.*, 2009). Their scanning electron micrograph of the serrate setae and the tubercles looked very similar compared to the ones described here for *N. granulata*. However, we propose that the setae are not stiff enough to act as a plectrum in *N. granulata*.

In two species of *Hemigrapsus* (Varunidae) from New Zealand and Chile, although no acoustic data were assessed, a descriptive approach of the proposed stridulating system structures was provided (McLay *et al.*, 2011). It was a "stridulating ridge" composed by the suborbital ridge and the distal inferior margin of the cheliped merus presenting an acute corneous ridge (plectrum) that engages the ridge when the merus is held against the pterygostomial area (McLay *et al.*, 2011). This stridulating system and type of movement is similar to the one described here for *N. granulata*. However, in the *Hemigrapsus* species, the plectrum was only found in males, thus, authors hypothesize that only males produce acoustic signals using this mechanism.

Similarly, the stridulating system of *Perisesarma* crabs was different between sexes: the presence of wear lines on the tubercles was only found in males, and females had few developed setae (Boon et al., 2009). Also, in species of Helice and Helicana (Varunidae) the stridulating system showed sexual dimorphism in the suborbital ridge: males have modified fused tubercles in the pars stridens and a prominent meral crest as a plectrum, while females have normal tubercles and lack a meral crest (Sakai et al., 2006). Here, we found that both sexes present a similar micromorphology of the pars stridens, although the plectrum of the males had prominent rounded tubercles whilst in the females, they were conical and less evident. Moreover, only males produced the sound signals registered in the present study. Thus, we suggest that under this reproductive scenario only males display acoustically by using the stridulating system described. However, the diverse setae type and tubercles of the pterygostomial area and merus in both sexes may indicate that these structures could be used in the production of other acoustic signals by either sex in different social contexts, not assessed in the present study. Additional studies are needed to elucidate different sound signals and structures involved in both sexes.

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- Abele, L. G., Robinson, M. H., and Robinson, B. (1973). "Observations on sound production by two species of crabs from panama (Decapoda, Gecarcinidae and Pseudothelphusidae)," Crustaceana 25, 147–152.
- Boon, P. Y., Yeo, D. C. J., and Todd, P. A. (2009). "Sound production and reception in mangrove crabs *Perisesarma* spp. (Brachyura: Sesarmidae)," Aquat. Biol. 5, 107–116.
- Budelmann, B. U. (1992). "Hearing in crustacea," in *The Evolutionary Biology of Hearing*, edited by D. B. Webster, R. R. Fay, and A. N. Popper (Springer-Verlag, Berlin), pp. 131–139.
- Buscaino, G., Filiciotto, F., Gristina, M., Bellante, A., Buffa, G., Di Stefano, V., Maccarrone, V., Tranchida, G., Buscaino, C., and Mazzola, S. (2011a). "Acoustic behaviour of the European spiny lobster *Palinurus elephas*," Mar. Ecol. Prog. Ser. 441, 177–184.
- Buscaino, G., Filiciotto, F., Gristina, M., Buffa, G., Bellante, A., Maccarrone, V., Patti, B., and Mazzola, S. (2011b). "Defensive strategies of European spiny lobster *Palinurus elephas* during predator attack," Mar. Ecol. Prog. Ser. 423, 143–154.

- Buscaino, G., Gavio, A., Galvan, D., Filiciotto, F., Maccarrone, V., de Vincenzi, G., Mazzola, S., and Orensanz, J. M. (2015). "Acoustic signals and behaviour of *Ovalipes trimaculatus* in the context of reproduction," Aquat. Biol. 24, 61–73.
- Clayton, D. (**2005**). "Substrate (acoustic/vibrational) communication and ecology of the ghost crab *Ocypode jousseaumei* (Brachyura: Ocypodidae)," Mar. Freshw. Behav. Physiol. **38**, 53–70.
- Clayton, D. (2012). "Acoustic calling in four species of ghost crabs: *Ocypode jousseaumei*, *O. platytarsus*, *O. rotundata* and *O. saratan* (Brachyura: Ocypodidae)," Bioacoustics 12, 37–55.
- Davie, P. J. F., and Ng, N. K. (2007). "Two new subfamilies of Varunidae (Crustacea: Brachyura), with description of two new genera," Raff. Bull. Zool. 16, 257–272.
- Dumortier, B. (**1963**). "Morphology of sound emission apparatus in Arthropoda," in *Acoustic Behavior of Animals*, edited by R. G. Busnel (Elsevier, Amsterdam), pp. 277–345.
- Field, L. H., Evans, A. E., and Macmillan, D. L. (1987). "Sound production and stridulatory structures in hermit crabs of the genus *Trizopagurus*," J. Mar. Biol. Assoc. U. K. 67, 89–110.
- Filiciotto, F., Sal Moyano, M. P., Hidalgo, F., de Vincenzi, G., Bazterrica, M. C., Ceraulo, M., Corrias, V., Quinci, E. M., Lorusso, M., Mazzola, S., Buscaino, G., and Gavio, M. A. (2019). "Underwater acoustic communication during the mating behaviour of the semi-terrestrial crab *Neohelice granulata*," Sci. Nat. 106, 35.
- Filiciotto, F., Sal Moyano, M. P., de Vincenzia, G., Hidalgo, F., Sciacca, V., Bazterrica, M. C., Corrias, V., Lorusso, M., Mazzola, S., Buscaino, G., and Gavio, M. A. (2018). "Are semiterrestrial crabs threatened by human noise? Assessment of behavioural and biochemical responses of *Neohelice* granulata (Brachyura, Varunidae) in tank," Mar. Poll. Bull. 137, 24–34.
- Guinot, D., Ng, N. K., and Rodríguez Moreno, P. A. (2018). "Review of grapsoid families for the establishment of a new family for *Leptograpsodes* Montgomery, 1931, and a new genus of Gecarcinidae H. Milne Edwards, 1837 (Crustacea, Decapoda, Brachyura, Grapsoidea MacLeay, 1838)," Zoosystema 40, 547–604.
- Guinot-Dumortier, D., and Dumortier, B. (1960). "La stridulation chez les crabes" ("Stridulation in crabs"), Crustaceana 1, 117–155.
- Horch, K. (1971). "An organ for hearing and vibration sense in the ghost crab, *Ocypode*," Z. Vgl. Physiol. 73, 1–21.
- Horch, K. (1975). "The acoustic behaviour of the ghost crab Ocypode cordimana Latreielle, 1818 (Decapoda, Brachyura)," Crustaceana 29, 193–204.
- Horch, K., and Salmon, M. (**1969**). "Production, perception and reception of acoustic stimuli by semi-terrestrial crabs (Genus *Ocypode* and *Uca*, family Ocypodidae)," Forma Functio. **1**, 1–25.
- Imafuku, M., and Ikeda, H. (1990). "Sound production in the land hermit crab *Coenobita purpureus* Stimpson, 1858 (Decapoda, Coenobitidae)," Crustaceana 58, 168–174.
- Iribarne, O., Bortolus, A., and Botto, F. (1997). "Between-habitat differences in the borrow characteristics and trophic modes in the Southwestern Atlantic borrowing crab *Chasmagnathus granulata* (Brachyura: Grapsoidea: Varunidae)," Mar. Ecol. Prog. Ser. 155, 137–145.
- López Greco, L. S., and Rodríguez, E. M. (1998). "Size at the onset of sexual maturity in *Chasmagnathus granulata* Dana, 1851 (Grapsidae, Sesarminae): A critical overall view about the usual criteria for its determination," *Crust. Biodivers. Crisis. Proc. Fourth Internat. Crust. Congr.* (Brill, Leiden, the Netherlands), pp. 675–689.
- McLay, C. L. (1988). *Brachyura and crab-like Anomura of New Zealand* (University of Auckland Bindery, Auckland).
- McLay, C. L., Hinnendael, F., Lavery, S., and Riquelme-Bugueño, R. B. (2011). "Morphological and molecular comparison of *Hemigrapsus crenulatus* (Milne Edwards, 1837) (Brachyura: Varunidae) from New Zealand and Chile: Was Miss Rathbun right?," J. Crust. Biol. 31, 582–589.
- Mowles, S. L., Jennions, M., and Backwell, P. R. (2017). "Multimodal communication in courting fiddler crabs reveals male performance capacities," R. Soc. Open Sci. 4, 161093.
- Parker, A. R., Mckenzie, D. R., and Ahyong, S. T. (1998). "A unique form of light reflector and the evolution of signaling in *Ovalipes* (Crustacea: Decapoda: Portunidae)," Proc. R. Soc. B. 265, 861–867.
- Patek, S. N. (2001). "Spiny lobsters stick and slip to make sound," Nature 411, 153–154.
- Patek, S. N., Shipp, L. E., and Staaterman, E. R. (2009). "The acoustics and acoustic behavior of the California spiny lobster (*Panulirus interruptus*)," J. Acoust. Soc. Am. 125, 3434–3443.

- Popper, A. N., Salmon, M., and Horch, K. W. (2001). "Acoustic detection and communication by decapod crustaceans," J. Comp. Physiol. A. 187, 83–89.
- Roberts, L., Harding, H. R., Voellmy, I., Bruintjes, R., Simpson, S. D., Radford, A. N., Breithaupt, T., and Elliott, M. (2016). "Exposure of benthic invertebrates to sediment vibration: From laboratory experiments to outdoor simulated pile-driving," Proc. Mtgs. Acoust. 27, 1–9.
- Sakai, K., Türkay, M., and Yang, S. L. (2006). "Revision of the *Helice*/ *Chasmagnathus* complex (Crustacea: Decapoda: Brachyura)," Abhan. Sencken. Naturfo. Gesels. 565, 1–77.
- Salmon, M. (**1965**). "Waving display and sound production in the courtship behavior of *Uca pugilator*, with comparisons to *U. minax* and *U. pugnax*," Zoologica **50**, 123–149.
- Salmon, M. (1967). "Coastal distribution, display and sound production by Florida fiddler crabs (Genus *Uca*)," Anim. Behav. 15, 449–459.
- Salmon, M., and Atsaides, S. P. (**1968**). "Visual and acoustical signalling during courtship by fiddler crabs (genus *Uca*)," Am. Zool. **8**, 623–639.
- Salmon, M., and Horch, K. (1972). "Acoustic signalling and detection by semi-terrestrial crabs of the family Ocypodidae," in *Recent Advances in the Behaviour of Marine Organisms*, edited by H. E. Winn, and B. Olla (Plenum, New York), pp. 60–96.
- Salmon, M., and Horch, K. (1973). "Vibration reception by the fiddler crab, Uca minax," Comp. Biochem. Physiol. 44, 227–241.
- Sal Moyano, M. P., Gavio, M. A., and Luppi, T. (2016a). "Different sperm allocation strategies in two populations of the semiterrestrial crab *Neohelice granulata* (Brachyura, Grapsoidea, Varunidae)," Mar. Ecol. 37, 737–749.
- Sal Moyano, M. P., Gavio, M. A., and Luppi, T. A. (2012a). "Mating system of the burrowing crab *Neohelice granulata* (Brachyura: Varunidae) in two

contrasting environments: Effect of burrow architecture," Mar. Biol. 159, 1403–1416.

- Sal Moyano, M. P., Gavio, M. A., McLay, C., and Luppi, T. A. (2014b).
 "Habitat-related differences in the pre-copulatory guarding and copulation behavior of *Neohelice granulata* (Brachyura, Grapsoidea, Varunidae),"
 J. Sea Res. 87, 8–16.
- Sal Moyano, M. P., Lorusso, M., Nuñez, J., Ribeiro, P., Gavio, M. A., and Luppi, T. (2016b). "Male size-dependent dominance for burrow holding in the semiterrestrial crab *Neohelice granulata*: Multiple tactics of intermediate-sized males," Behav. Ecol. Soc. 70, 1497–1505.
- Sal Moyano, M. P., Luppi, T., Gavio, M. A., Vallina, M., and McLay, C. (2012b). "Receptivity of female *Neohelice granulata* (Brachyura, Varunidae): Different strategies to maximize their reproductive success in contrasting habitats," Helgol. Mar. Res. 66, 661–674.
- Sal Moyano, M. P., Silva, P., Luppi, T., and Gavio, M. A. (2014a). "Female mate choice by chemical signals in a semi-terrestrial crab," J. Sea Res. 85, 300–307.
- Schmitt, W. L. (1965). *Crustaceans* (The University of Michigan Press, Ann Arbor, Michigan).
- Seiple, W., and Salmon, M. (1982). "Comparative social behavior of two grapsid crabs, *Sesarma reticulatum* (Say) and *S. cinerum* (Bosc)," J. Exp. Mar. Biol. Ecol. 62, 1–24.
- Spivak, E. (2010). "The crab *Neohelice* (=*Chasmagnathus*) granulata: An emergent animal model from emergent countries," Helgol. Mar. Res. 64, 149–154.
- Takeshita, F., and Murai, M. (2016). "The vibrational signals that male fiddler crabs (*Uca lactea*) use to attract females into their burrows," Sci. Nat. 103, 49.
- Taylor, J. R. A., and Patek, S. N. (2009). "Crustacean seismic communication: Heard but not present?," in *The Use of Vibrations in Communication: Properties, Mechanisms and Function Across Taxa*, edited by C. E. O'Connell-Rodwell (Research Signpost, Kerala, India), pp. 9–24.