

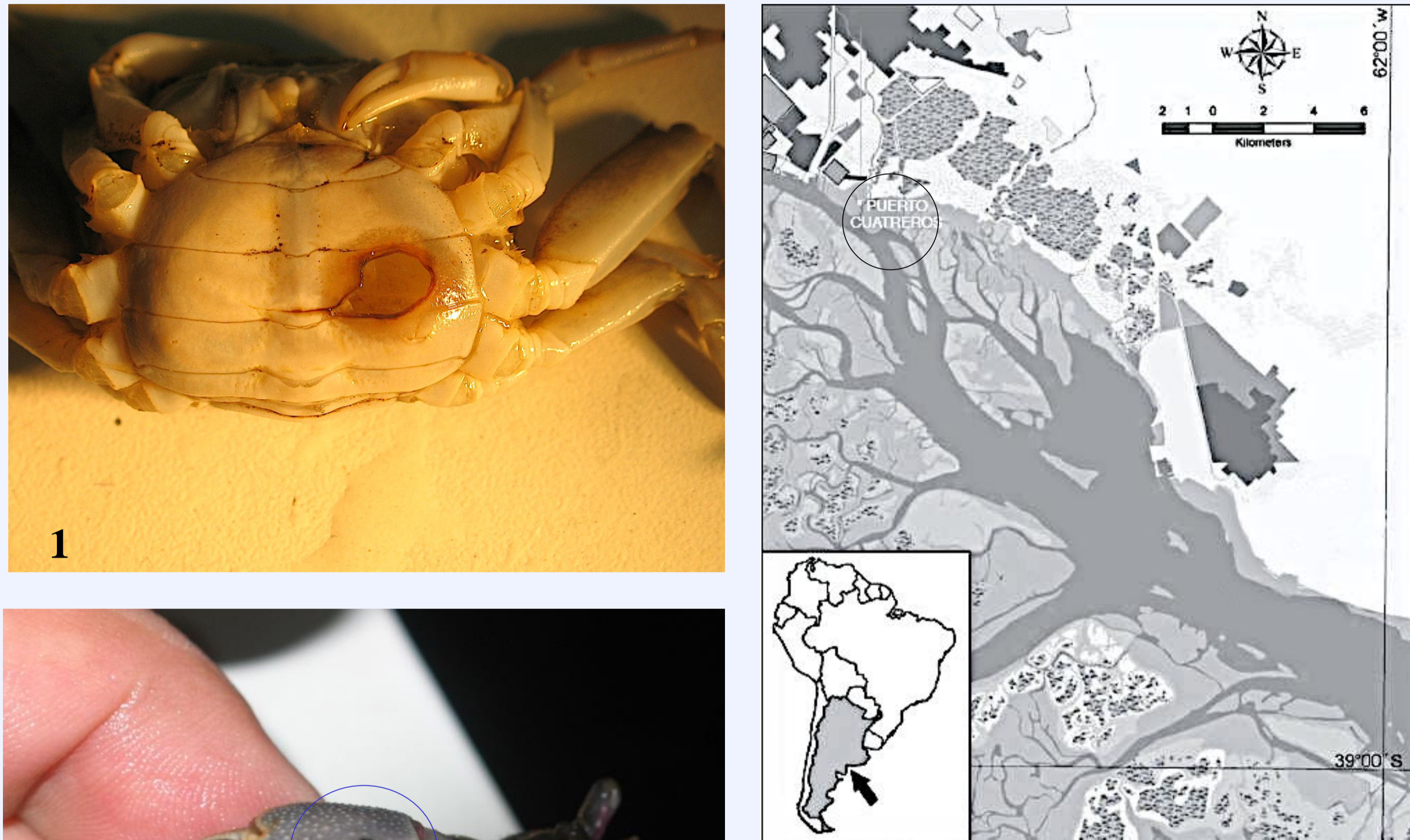
Shell disease in *Cyrtograpsus Angulatus* and *neohelice granulata*, grapsid crabs from Bahía Blanca estuary, Argentina

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In Argentina, Atlantic salt marshes are inhabited mainly by abundant populations of two grapsid crabs *Cyrtograpsus angulatus* Dana, 1851 and *Neohelice granulata* (Dana, 1851). In a survey carried out to increase the knowledge about pathogens of crustaceans in Argentina, these two crab species were studied in the Bahía Blanca estuary. This estuary is the second largest in Argentina, after the Río de La Plata estuary. It is characterized by extensive tidal flats, a complex system of tidal channels, patches of low salt marshes, and shallow islands.

The Bahía Blanca estuary



METHODS

Crabs were collected from the innermost part of the estuary. in Puerto Cuatrerros (38°44S 62°22W) using hand nets and foldable crab traps. The specimens were transported to the laboratory alive and some crabs with lesions were fixed in 10% formalin for histological studies. In the laboratory lesions from live crabs were scraped in aseptic conditions and the material was spread in plates with TCBS and incubated at room temperature for 24-48 hs. DNA was extracted from colonies using DNeasy kits (Quiagen). PCR amplification was conducted using illustra PuReTaq Ready-To-Go™ PCR Beads (GEHealthcare) and general primers for 16s rDNA were used. PCR products were then sequenced using the MacroGen® service.

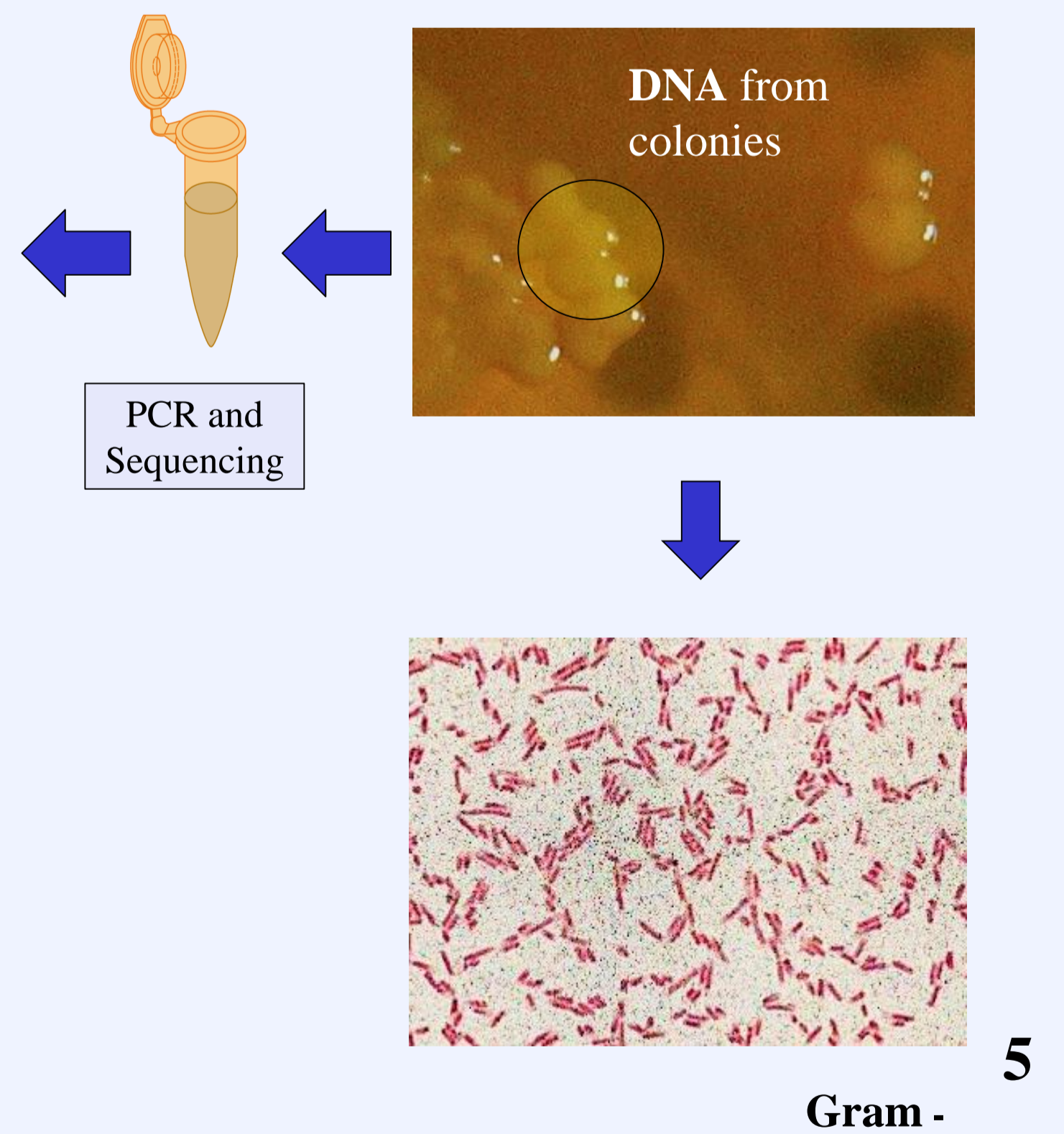
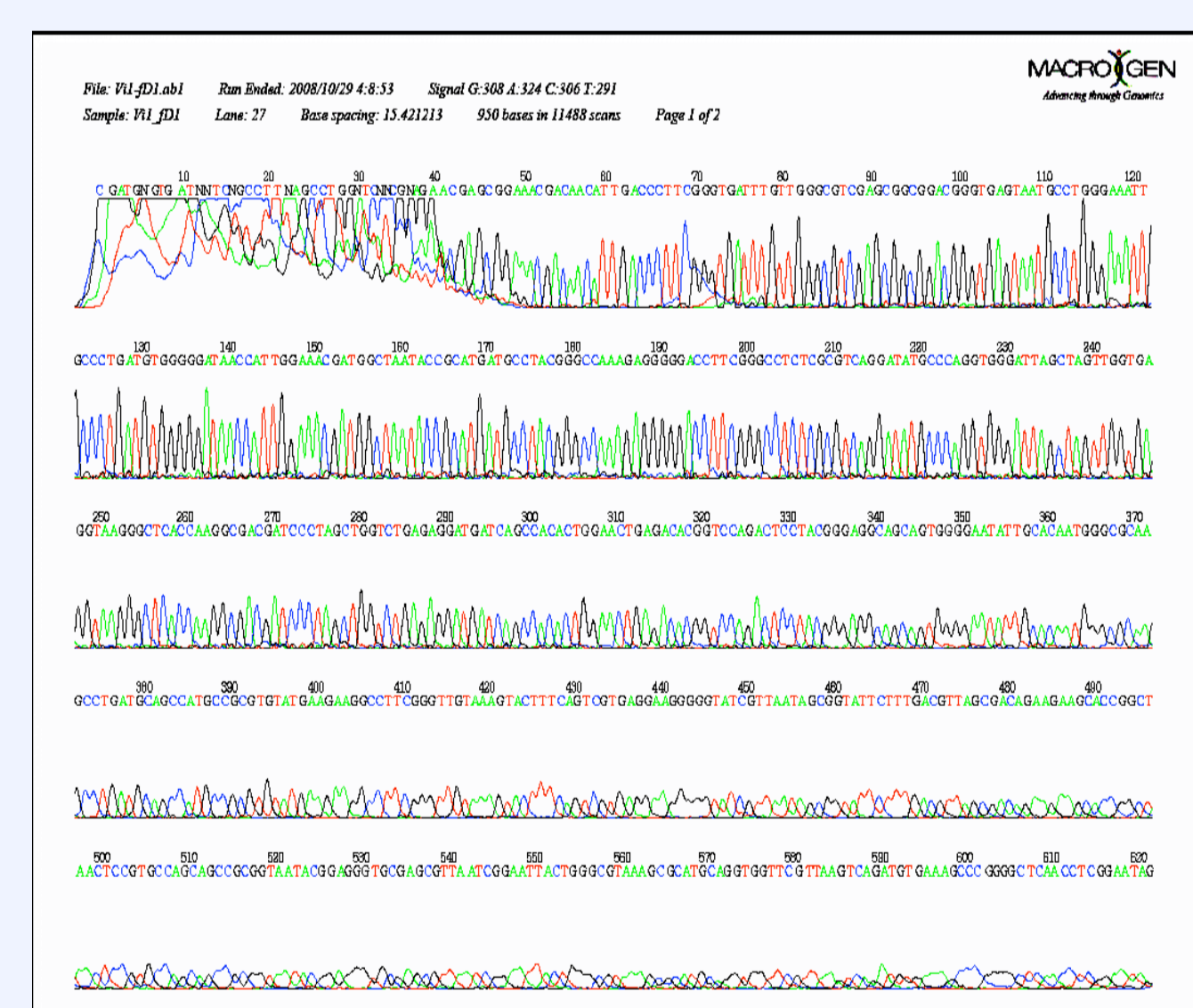
RESULTS AND CONCLUSIONS

In 5% of the grapsid crabs *Neohelice granulata* (n=36) and *Cyrtograpsus angulatus* (n=65) collected in Bahía Blanca estuary, erosive lesions (up to 5 mm diameter) were observed in ventral and dorsal surfaces of shells.

In histological sections of the cuticle very extensive foci were observed (Fig.3). Lesions were characterized by extended erosions throughout the epi-, exo-, and endocuticle, wich was often melanized. In the foci, bacteria, protozoans, and debris were observed.

Positive plates in TCBS (Fig. 4) showed round, yellow, and bright colonies integrated by Gram-negative bacteria (Fig. 5). DNA extracted from this colonies was amplified by PCR and sequenced. DNA sequences were compared to the ones deposited in GenBank (BLASTN) and the Ribosomal Database Project (RDP). We observed a close relationship with several *Vibrio* spp. Some of these species have been reported in association with crustacean culture: *V. alginolyticus* (96% BLASTN), *V. furnissii* (95% BLASTN), *V. vulnificus* (96% BLASTN), *V. fluvialis* (95% BLASTN), *V. paccinii* (85% RDP), and *V. parahaemolyticus* (86% RDP).

Biochemical reactions will be necessary for the final species identification of the causative agent of this shell disease.



Accession	Species	Gene	Length	Identity	Score	E-value	Bit score
AY654796.1	<i>Mycobacterium</i>	7 16S ribosomal RNA gene, partial sequence	1452	1452	95%	0.0	95%
AY654751.1	<i>Mycobacterium</i>	13 16S ribosomal RNA gene, partial sequence	1452	1452	95%	0.0	95%
EU539397.1	Uncultured bacterium clone nbt238b12	16S ribosomal RNA gene, partial sequence	1450	1450	90%	0.0	96%
DQ647617.1	<i>Vibrio</i> sp.	99WF10-51 16S ribosomal RNA gene, partial sequence	1450	1450	91%	0.0	96%
AY654827.1	<i>Mycobacterium</i>	98 16S ribosomal RNA gene, partial sequence	1450	1450	95%	0.0	95%
AB457056.1	<i>Vibrio</i> sp.	BB-11 gene for 16S ribosomal RNA, partial sequence	1448	1448	95%	0.0	95%
DQ664544.1	<i>Vibrio alginolyticus</i> strain RH2	16S ribosomal RNA gene, partial sequence	1448	1448	91%	0.0	96%
DQ159098.1	<i>Bacterium</i> PRR-A6	16S ribosomal RNA gene, partial sequence	1448	1448	91%	0.0	96%
AF513447.1	<i>Vibrio alginolyticus</i>	16S ribosomal RNA gene, partial sequence	1448	1448	91%	0.0	96%

***Vibrio alginolyticus* 96 % BLAST**
***Vibrio vulnificus* 96 % BLAST**



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