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Taxonomic reassessment of *Tetrapygus niger* (Arbacioida, Echinoidea): molecular and morphological evidence support its placement in *Arbacia*

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Abstract

The echinoid genus *Tetrapygus* was initially described by L. Agassiz (1841) based on a single species, *Tetrapygus niger* Molina, 1782. Since the extensive work conducted by Mortensen (1935), *Tetrapygus* has received limited taxonomic attention over the past century. Recent discoveries of new fossil species of *Arbacia* Gray, 1835 from the upper Pliocene of northern Chile revealed striking morphological similarities between the two distinct Arbaciidae genera *Arbacia* and *Tetrapygus*. These findings compelled new investigations to evaluate the taxonomic status of these genera. Based on molecular mitochondrial (COI), nuclear (28S), and morphological evidence, *Tetrapygus niger* is here recovered as the sister species to *Arbacia dufresnii*, both species forming a clade within the phylogeny of South American species of *Arbacia*. Consequently, the diagnosis and description of *Tetrapygus niger* are here revised, and the species is reattributed to *Arbacia*, as previously proposed by A. Agassiz in Agassiz & Desor (1846) under the species name *Arbacia nigra*. An emended diagnosis of *Arbacia* is also proposed in light of these new findings.

Key words: Echinoidea, Arbaciidae, *Tetrapygus*, *Arbacia*, Taxonomy

Introduction

Tetrapygus L. Agassiz, 1841 is a monospecific genus of the family Arbaciidae. It is one of the common shallow-water representatives of this family along with the two other genera, *Coelopleurus* L. Agassiz, 1840 and *Arbacia* Gray, 1835. *Tetrapygus niger* Molina, 1782 is a conspicuous species (Rodríguez 2003) occurring along the temperate east Pacific coasts from northern Peru to south-central Chile (Puerto Montt) (Haye *et al*. 2014; Mortensen 1935; Rodríguez & Ojeda, 1993). The species inhabits shallow subtidal to upper sublittoral environments. Although initially reported between 0 and 10 m depth (Mortensen 1935), it is now known to occur down to 40 m (Larraín 1975). Recent research has also reported the presence of the species for the first time in mesophotic environments off central Chile at a depth of 47.6 m (Campoy *et al.* 2023). However, it is more commonly found between 0 and 10 m, thriving in shallow rocky ecosystems with densities reaching up to 85 individuals/m^2 (Rodríguez 2003; Rodríguez & Ojeda 1993). The species is well-known for its significant impact on the distribution of *Lessonia trabeculata*,

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Villouta & Santelices, 1986 one of the most common kelp species found along the Chilean coast (Perreault *et al.* 2014; Rodríguez 2003). Along the coasts of Peru, population outbreaks in kelp forests can lead to overgrazing and the development of extensive barren grounds (Hooker *et al.* 2013). Additionally, a large population of *Tetrapygus niger* was discovered in an oyster farm in Alexander Bay, South Africa, suggesting potential invasive dynamics of the species (Filander & Griffiths 2014). Otherwise, *Tetrapygus niger* is primarily considered an omnivorous species, with a diet mainly consisting of calcareous algae.

Tetrapygus niger has been extensively studied in toxicology and trophic ecology, as evidenced by over 200 studies published in the last five years (analysis of Google Scholar entries accessed on 07.17.2023 with query: "*Tetrapygus niger*"). Haye *et al.* (2014) conducted research in phylogeography, revealing high dispersal capacity of the species. However, studies focusing on genetic relationships among the Arbaciidae are relatively few, and *Tetrapygus* is typically excluded from large-scale molecular analyses of echinoids (Mongiardino-Koch *et al.* 2018; Smith *et al.* 2006). Consequently, relationships within the Arbaciidae remain unclear. *Tetrapygus* was previously considered a suitable outgroup in the phylogeny of *Arbacia* (Lessios *et al.* 2012). Nevertheless, the recent discovery of new fossil species of *Arbacia* from the upper Pliocene of Chile sharing morphological characters with both *Arbacia* and *Tetrapygus* raised the need for further taxonomic investigations of the two genera (Courville *et al.* 2023). The objectives of the present study are: (i) to revise the systematics of *Tetrapygus niger*, (ii) to elucidate phylogenetic relationships among shallow-water representatives of the Arbaciidae, including *Tetrapygus*, *Arbacia*, and *Coelopleurus*, and (iii) to revise the taxonomic status and diagnostic features of *Arbacia* and *Tetrapygus.*

Materials and methods

Material studied. New material of *Tetrapygus niger* was collected in Concepción by Angie Díaz and Erwan Courville in May 2022 (Fig. 1). In addition, an extensive collection from intertidal zones at various localities from Chiloe (South Chile) to Lima (Peru) between September 2009 and July 2012 was examined and sequenced (FONDECYT 1090670; Haye *et al.* 2014). Tissue samples from two *Arbacia spatuligera* Valenciennes, 1846, collected off Concepción by Angie Díaz (VRID Initiation 217.113.093-1 OIN; ANID-Millenium Science Initiative Program; ICN2021_002; PIA CONICYT ACT172065) and three specimens collected by Thomas Saucède from Coquimbo (Chile; ASSEMBLE Grant No. 22779) are currently housed at the Molecular Ecology Laboratory (LEM), Faculty of Ecology Sciences, Universidad de Chile, Santiago, Chile, and Universidad de Concepción, Concepción, Chile. Furthermore, six specimens of *Arbacia dufresnii* Blainville, 1825 collected in Punta Arenas in 2022 were used and are stored at the LEM, Santiago Chile. A tissue sample from a *Coelopleurus floridanus* A. Agassiz, 1872 collected off the coast of French Guiana and stored at the University of Florida was also sequenced. The analysis was complemented by the examination of the neotype material of *T. niger* defined by Larraín (1975) and housed at the Museum of Zoology, Universidad de Concepción, Chile (MZUC-UCCC n° 7967).

Extraction and sequencing. The isolation of DNA, PCR, and preparation for Sanger sequencing were performed at the LEM, Universidad de Chile, Chile. DNA was extracted from the muscles attached to the spines and gonads preserved in 95% ethanol using the Dneasy Blood & Tissue Kit by QIAGEN. The Cytochrome Oxidase subunit I (COI) of the mitochondrial genome was amplified for ten individuals of *T. niger*, six *A. dufresnii*, five *A. spatuligera*, and one *C. floridanus*. COI primers specific for *Arbacia* were used, ArbaF and ArbaR (Haye *et al.* 2014). The 28S of the nuclear genome was amplified for four specimens of *T. niger*, two *A. dufresnii*, two *A. spatuligera*, and one *C. floridanus* (see Table 1 for details). Internal and external primers for 28S, designed by Stockley *et al.* (2005), were employed. PCRs were performed using Accustart II PCR ToughMix (ref. 95142-800). For a 50 µl PCR reaction, 5 µl of total genomic DNA (not quantified), 1 µl of each primer (0.2 µM) and 25 µl of Taq polymerase were used. Cycling conditions consisted of an initial denaturation at 94°C for 3 min followed by 40 cycles of 1 min denaturing at 94°C, 45 sec annealing at 45°C for COI, 45 sec annealing at 55°C for 28S, and a 1 min extension at 72°C, with a final 3min extension at 72°. We sequenced in one direction, using automatic sequencers at MACROGEN Korea and Chile.

Phylogenetic analysis. To avoid the possibility that sequences of pseudogenes were included in the mitochondrial DNA, ambiguous chromatograms were discarded, and the remainder were translated to amino acids to check for stop codons using MEGA 11 version 11 (Tamura *et al.* 2021). Sequence contigs were aligned by eye using the software ProSeq v2.91 (Filatov 2002). Kimura 2-parameter distances (K2P) were calculated and generated using MEGA 11.

Bayesian inference of phylogeny (BI) were performed with MrBayes v.3.2.6 (Ronquist & Huelsenbeck 2003) as implemented in NGPhylogeny.fr server (Lemoine *et al*. 2019) using the best fitted GTR+Γ+I model. BI tree was inferred with 10^7 MCMC generations with tree sampling every 1000 generations and a burn-in of 25%. A Maximum Likelihood (ML) tree was inferred with PhyML v3.1 (Guindon *et al*. 2010) as implemented in NGPhylogeny.fr with GTR as the evolutionary model, discrete gamma model with categories (n = 4), empirical equilibrium frequencies, SPR (Subtree Pruning and Regraphting) tree topology search and approximate Bayes branch support.

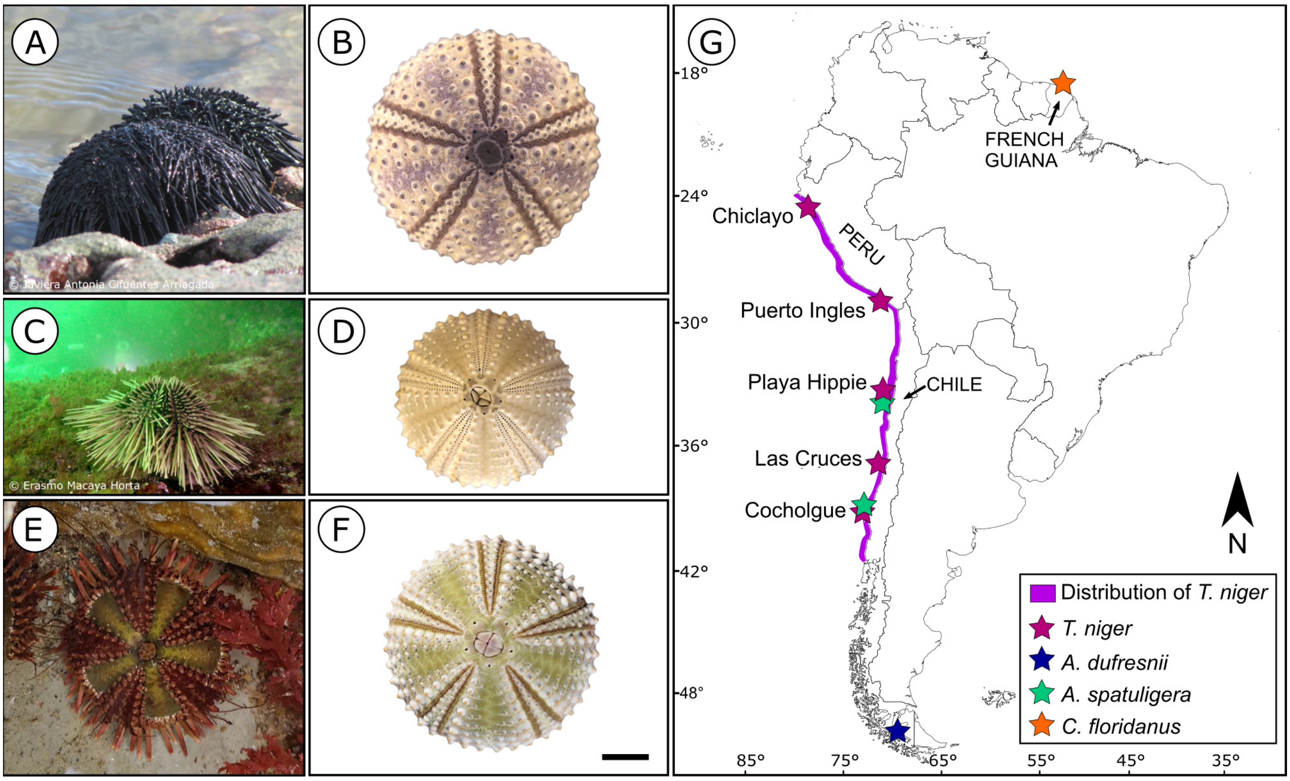

FIGURE 1. A–B, *Tetrapygus niger*; **C–D**, *Arbacia spatuligera*; **E–F**, *Arbacia dufresnii*; **G**, Distribution of studied Arbaciidae and sampling sites. Scale bar equals 10 mm.

Additional sequences of *Arbacia lixula* (Linné, 1758), the type species of *Arbacia*, were obtained from GenBank and are mentioned in Table 1. To root the phylogeny, sequences of one outgroup were chosen within the order Arbacioida (*Coelopleurus*).

Morphological study. To facilitate morphological observations, the specimens underwent a cleaning process using a brush dipped in a diluted bleach solution. This solution effectively removed all the spines and soft tissues, exposing the underlying structures for examination. The plating of the echinoid test was then drawn using a binocular microscope. In addition, pedicellariae and spines were carefully prepared by subjecting them to a diluted bleach treatment under a binocular microscope, followed by rinsing with water to halt the reaction. Pedicellariae and spines were dried, mounted on SEM stubs and studied with a tabletop scanning electron microscope so that digital images could be recorded.

Terminology. Systematic nomenclature and terminology for morphological description follow Kroh (2020). Plates are numbered according to Lovén's system (Lovén, 1874). In extant species of Arbaciidae, morphological variations during growth are important, making it difficult to assign the smallest specimens (below 30 mm in diameter) unequivocally to a given species (Kroh *et al*. 2012). Species descriptions here are based on examination of the largest specimens when available, for which morphological differences among species are more diagnostic.

TABLE 1. Material and GenBank accession numbers of taxa in this study.

Species	Location	n	Sample number	Coordinates	28S Accession Number	COI Accession Number	References
T. niger	Chiclayo (Peru)	2	TN_3CHI	6.80°S, 79.95°W	OR569206	OR569171	This work
			TN_4CHI			OR569172	
	Puerto Ingles (Peru)	2	TN_7ILO	17.60°S, 71.35°W	OR569205	OR569174	
			TN_10ILO		OR569203	OR569173	
	Playa Hippie (Chile)	2	TN_2PH	26.40°S, 70.70°W	OR569202	OR569175	
			TN_8PH		OR569204	OR569176	
	Las Cruces (Chile)	2	TN_31LC	33.49°S, 71.63°W		OR569177	
			TN_32LC			OR569178	
	Cocholgue (Chile)	2	TN_2CO	36.59°S, 72.98°W		OR569179	
			TN_8CO			OR569180	
A. dufresnii	Seno Otway (Chile)	2	AD_19SO	52.88°S,71.12°W		OR569181	This work
			AD_21SO			OR569184	
	Faro San Isidro (Chile)	3	AD_10FI	53.78°S, 70.97°W	OR569198	OR569182	
			AD_11FI			OR569185	
			AD_12FI			OR569186	
	Fuerte Bulnes (Chile)	1	AD_03BUL	53.62°S, 70.94°W	OR569199	OR569183	
A. spatuligera	Coquimbo (Chile)	3	AS_1COQ	29.97°S, 71.38°W		OR569187	This work
			AS_3COQ		OR569200	OR569188	
			AS_8COQ			OR569189	
	Concepción (Chile)	2	AS_4CONC	36.59°S, 72.98°W	OR569201	OR569190	
			AS_6CONC			OR569191	
A. lixula	Azores	3	NA	NA	DQ073753	JN603630-33	(Kroh *et al.* 2012; Smith *et al.* 2006)
C. *floridanus*	French Guiana	1	NA	NA	OR569207	OR569192	This work

Results

Phylogenetic relationships—The length of the COI section obtained for the species of *Arbacia*, *Tetrapygus* and *Coelopleurus* was 655 bp, and the length of the 28S was 469 bp. The sequences generated for this study were submitted to GenBank (Table 1), and are also found in https://doi.org/10.5281/zenodo.10085730 (Courville *et al.* 2023b). No stop codons, insertions, and deletions were observed in the COI sequences, indicating that they represent functional mitochondrial genes.

Bayesian inferences as well as Maximum Likelihood resulted in trees with similar topology in terms of branching and robustness. BI trees are represented in Figure 2 for COI (A) and 28S (B).

Both tree topologies (Fig. 2A, B) reveal the same relationship among all the included species. The species of *Coelopleurus* exhibit high genetic distances from all other Arbaciidae species (Tables 2–3). All tree topologies consistently demonstrate monophyletic groupings for all *Arbacia* species, with robust node supports. *Arbacia lixula* appears to be the sister-species of the subclade that includes all Pacific species, *A. dufresnii*, *A. spatuligera* as well as *Tetrapygus niger*. *T. niger* consistently emerges as the sister species of *A. dufresnii*, the subclade formed by the two species exhibiting strong node support.

Table 2 provides a summary of K2P genetic distances in COI among species. When comparing COI sequences of *T. niger* with *A. dufresnii*, a difference of approximately 2% is observed. In contrast, genetic distances between *A. dufresnii* and *A. spatuligera* are higher, exceeding 6%. The genetic distance between *T. niger* and *A. spatuligera* falls within an intermediate range, approaching 5.18%. Genetic distances within the clade that includes *A. spatuligera*, *A. dufresnii* and *T. niger,* and *A. lixula* reach 11% which is also consistent with expected mean intrageneric values among echinoids, 10.6% (Ward *et al.* 2008). Distances between the studied species of *Arbacia* and *C. floridanus* are up to approximately 22%.

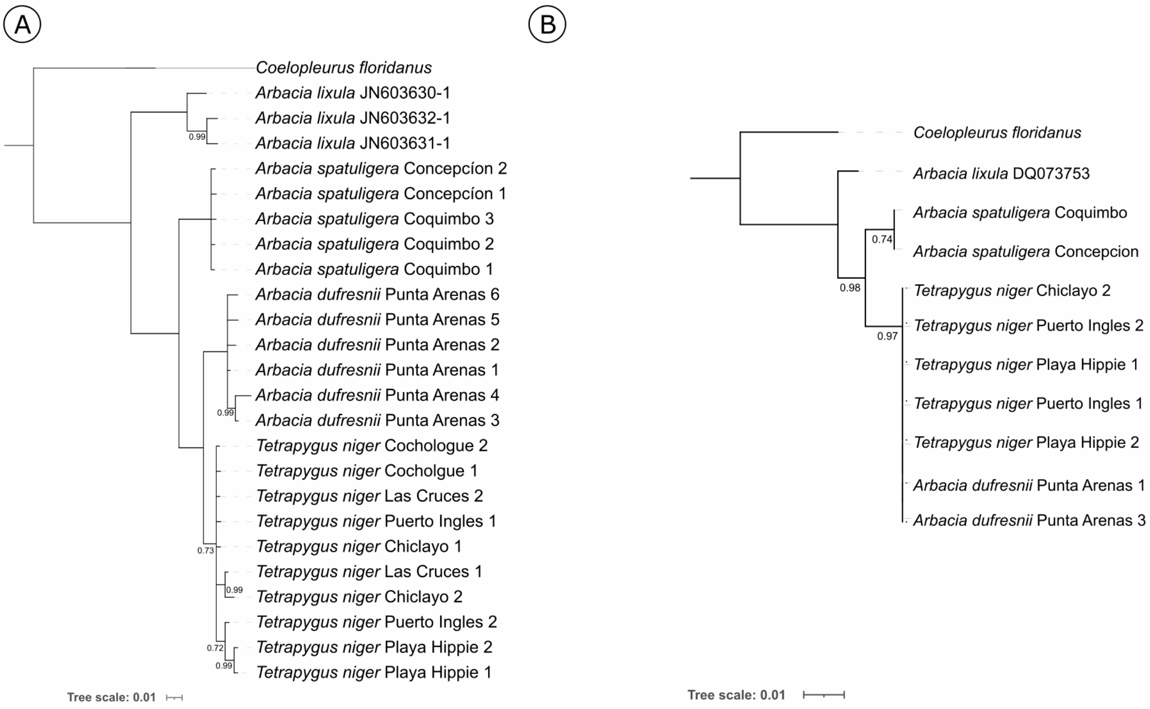

FIGURE 2. Relationships among some Arbaciidae. **A,** BI (Bayesian Inference) tree of COI sequences; **B,** BI tree of 28S sequences. Values under each node respectively correspond to BI posterior probabilities below 1.

TABLE 2. Summary of COI genetic divergences (K2P distances) among species and genera.

	A. dufresnii	*T. niger*	*A. spatuligera*	*A. lixula*
A. dufresnii				
T. niger	2.01%			
A. spatuligera	6.66%	5.30%		
A. lixula	11.47%	11.17%	11.18%	
C. floridanus	21.14%	19.70%	19.65%	20.96%

TABLE 3. Summary of 28S genetic divergences (K2P) between species and genus.

	A. dufresnii	*T. niger*	*A. spatuligera*	*A. lixula*
A. dufresnii				
T. niger	0.00%			
A. spatuligera	0.21%	0.21%		
A. lixula	0.67%	0.67%	0.45%	
C. floridanus	3.17%	3.17%	3.17%	3.56%

As expected, 28S sequences exhibit much lower values among species than those from COI (Table 3; Stockley *et al*. 2005). In addition, *A. dufresnii* and *T. niger* shared one haplotype, indicating their genetic identity for this marker. In contrast, *A. spatuligera* and *A. lixula* exhibited different haplotypes with 0.21 and 0.67% genetic distance respectively, while haplotypes of species of *Arbacia* all diverged by around 3% from *C. floridanus.*

This study, derived from the analysis of mitochondrial and nuclear markers, provides compelling evidence of the proximity of *T. niger* to *A. dufresnii*. The short genetic distance of less than 2% between COI sequences of the two species that also shared a common 28S haplotype demonstrate their close relationships. These results highlight the need of conducting a revision of the systematics of *T. niger* and of the taxonomic status of both genera based on the obtained evidence.

Systematic part

Phylum Echinodermata Klein, 1778

Class Echinoidea Schumacher, 1817

Order Arbacioida Gregory, 1900

Family Arbaciidae Gray, 1855

Genus *Arbacia* Gray, 1835

Type species. *Arbacia lixula* (Linné, 1758). Extant species with a wide distribution across the Atlantic Ocean, including the Brazilian coast, the Gulf of Guinea, the Azores, the East Atlantic Islands, and the Mediterranean Sea. The genus is also known from the Pleistocene of Madeira, Azores, and Italy (Madeira *et al.* 2011; Stefanini 1911).

Emended diagnosis. Arbaciidae with ocular plates bearing small tubercle, usually resorbed during ontogeny; periproct slightly elongated in direction 1-IV and covered with four supra-anal plates; ocular pores double, not visible externally; peristome large (> 30% TD), the ambulacra protruding so as to form "ambulacral lips"; buccal notches shallow and wide; tags narrow, more or less elongated; oral primary spines with a cortex-layer "shoe" or "cap"; the ambital and upper spines without any cortex-layer; sphaeridia single, in a small pit close to the peristomial edge; epistroma conspicuously developed and may be of a characteristic design, forming randomly distributed punctation, or arranged in horizontal or vertical series.

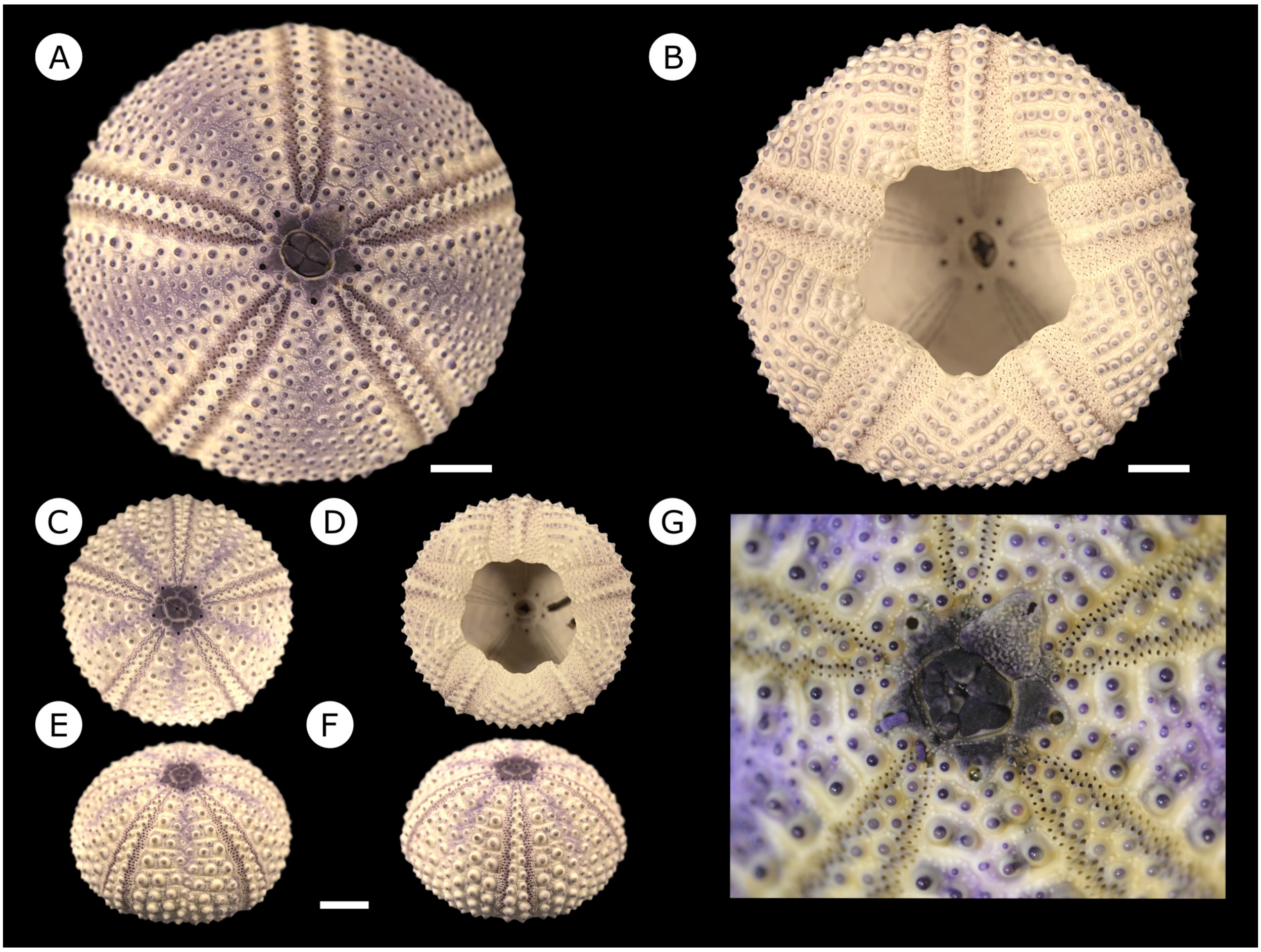

FIGURE 3. Denuded test of *Arbacia nigra* from Las Cruces (A–F; Chile) and Concepción (G; Chile). **A–B**, specimen TN_ 35LC: **A**, aboral view; **B**, oral view. **C–F**, specimen TN_9LC; **C**, aboral view; **D**, oral view; **E–F**, lateral views; **G**, apical system of specimen TN_2CO. Scale bars equal 10 mm.

Arbacia nigra (Molina, 1782)

1782 *Echinus niger* Molina, 1782 p. 175.
1841 *Tetrapygus niger* L. Agassiz: p. 7.
1863 *Arbacia nigra* A. Agassiz: p. 20.
1910 *Tetrapygus niger* (Molina) Mortensen: p. 35–36; fig. XV 12, 15.
1935 *Tetrapygus niger* (Molina) Mortensen: p. 582–584; fig. LXX 10–12; LXXXVII 15–19.

Emended diagnosis. *Arbacia* with ocular plate V invariably insert; ambulacral plates quadrigeminate to quinquegeminate; additional secondary tubercles below and above primary tubercles for each interambulacral plate above the ambitus; cleaned test deep purple; valves of aboral ophicephalous pedicellariae bilobed; epistroma well-developed, present as punctation among tubercles in ambulacra and interambulacra; tips of oral spines with vestigial "enameled" tip only.

Type material. Type specimen was not established in the original description, which also lacks illustrations. Larraín (1975) designated a neotype, housed in the Museum of the Zoological Department of the Universidad de Concepción, Chile, under catalogue number MZUC-UCCC n° 7967. The neotype was collected in Bahía Concepción, Chile (36°40'S, 73°W) in 1973. It is preserved dry, retaining all its spines and soft tissues, except for a cleaned ambulacrum, extending from the apical system to the peristome (TD = 77.3 mm; PD = 29.5 mm; TH = 39.2 mm).

Description. Test circular to subpentagonal in outline at ambitus. Test large, reaching 80 to 90 mm in diameter. Living specimens deep purple. Denuded test and spines purple. As for other species in genus, test height highly variable. Test height relative to test diameter increasing with size, allometric relationship between test height and test diameter described by linear regression model $\ln(y) = 1.2112\ln(x)—\ln(1.6139)$; $R^2 = 0.9612$.

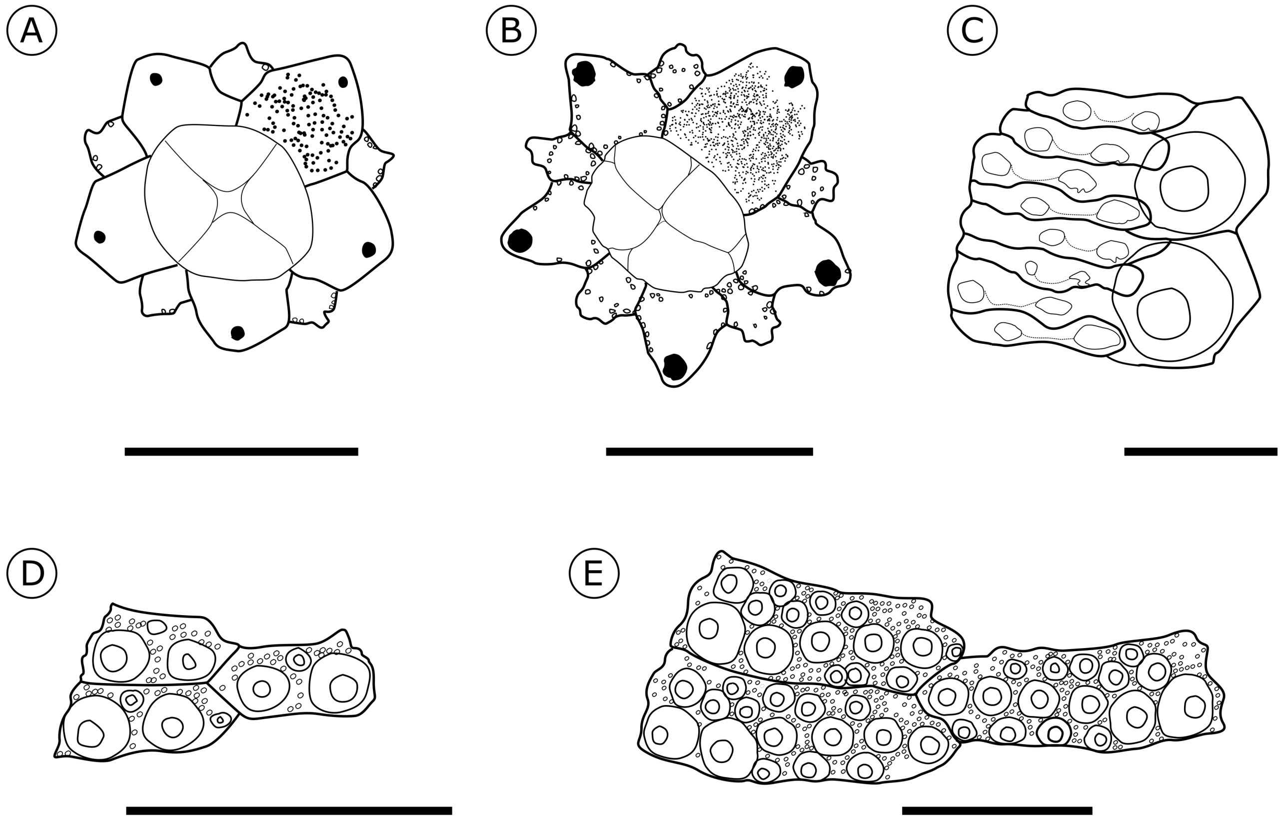

FIGURE 4. *Arbacia nigra*, details of test plating. **A–B**, apical system: **A**, small specimen (TD = 28 mm); **B**, large specimen (TD = 70 mm); **C**, aboral plates of ambulacrum; **D–E**, aboral plates of interambulacrum: **D**, small specimen (TD = 29 mm); **E**, large specimen (TD = 70 mm). Scale bars equal 10 mm.

Apical system—Small, hemicyclic, with ocular plate V always insert (Figs. 3A, C, G, 4A, B) and plates I and IV also often insert in adult, large specimens (oculars I, V, IV insert 57%, according to Jackson 1912). Juvenile always with dicyclic apical system, ocular plates becoming insert with growth. Ocular and genitals free of tubercles in adults, but juveniles have vestigial tubercle on each ocular as do other species of genus. Ocular plates bearing ophicephalous pedicellariae. Genital plates and periproct surrounded by pedicellariae. Periproctal membrane with four anal plates, purple, with a white adoral band (Fig. 3A, C). Periproct oval, with maximum diameter in direction 1-IV.

Peristome—Relative peristome diameter less than in other *Arbacia* species, usually close to 35% of test diameter. Relative peristome size decreasing with growth. Buccal notches shallow. Tags long and narrow (spanning >2 tubercles). Perignathic girdle large, auricles in contact, sometimes fused with each other, forming arch over perradius. Allometric relationship between peristome diameter and test diameter described by linear regression model $\ln(y) = 0.8094\ln(x) + \ln(0.0714)$; $R^2 = 0.968$.

Ambulacra—Very narrow (one third width of interambulacra), each plate with one imperforate and non-crenulate primary tubercle of almost same size as interambulacral ones at the ambitus and progressively decreasing in size adapically (Figs. 3A, F, 4C). Above ambitus, ambulacral tubercles smaller than interambulacrals. Tubercles closely spaced, occupying most of each ambulacral plate, separated from each other by few small granules perradially (Fig. 3F). Tubercles small, forming two well-developed columns of tubercles reaching apical system (Fig. 3A). Ambulacral plates quadrigeminate to quinquegeminate, of arbaciid type aborally (Fig. 4C), forming more complex compound plating adorally, leading to wide phyllodes occupying entire oral side adorally (Fig. 3B, D). Close to peristome, single perradial pit for sphaeridium along perradius of each ambulacrum (Fig. 3B).

Ambulacral pores—As in all *Arbacia* species, oral and aboral pores differentiated. Aborally, pores round and deeply conjugate. These conjugate isopores correspond to Smith's C1-type classification (Smith, 1978). Oral pores exhibit morphological features characteristic of Smith's P4 type (Smith 1978), including large circular interporal partition and broad circular attachment area. Tube feet, associated with small pores inside large periporal areas, ending in large adhesive discs with thick connective tissue, allowing for strong fixation to hard substrates.

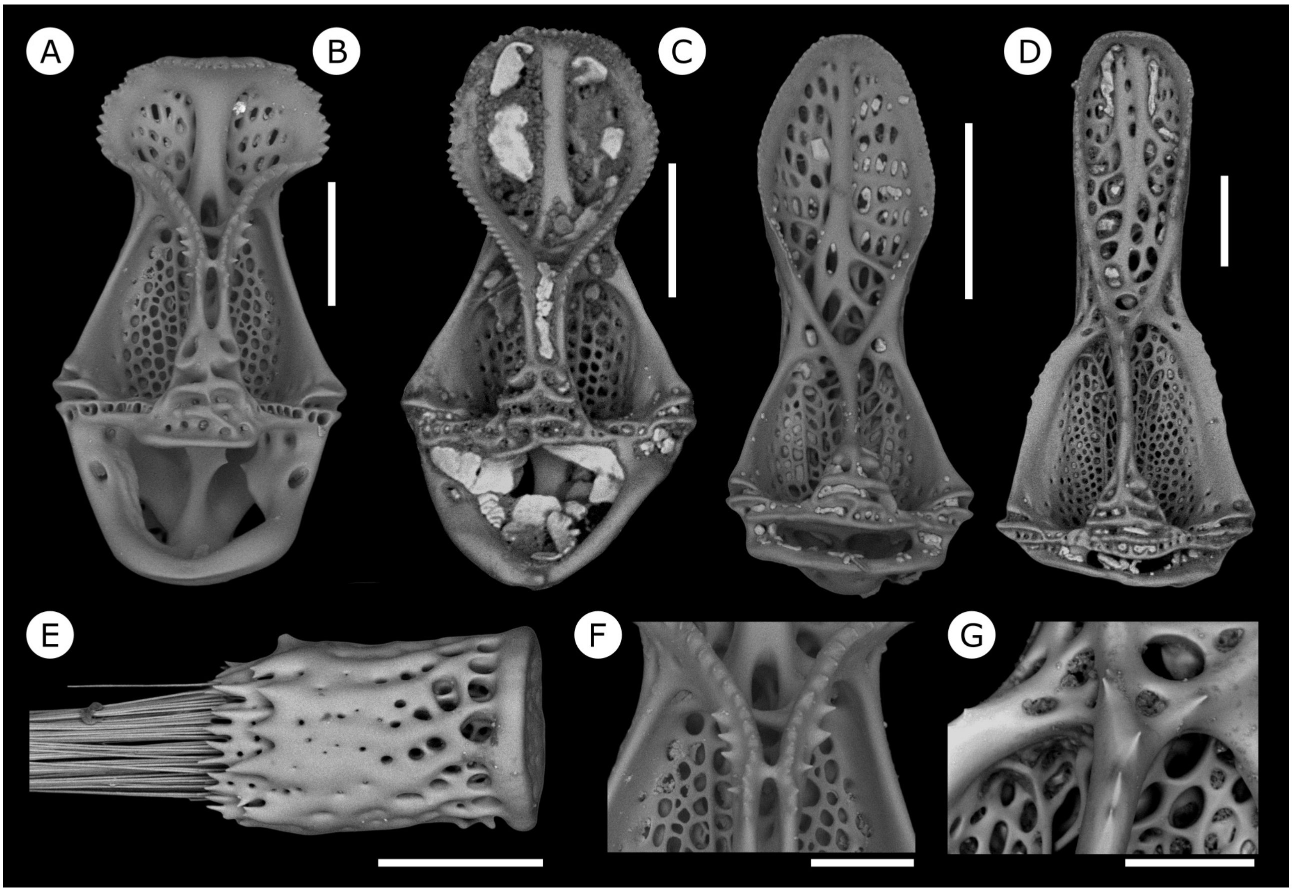

FIGURE 5. **A–G**, SEM photographs of pedicellariae and stalk of *Arbacia nigra*; **A**, aboral ophicephalous pedicellaria; **B**, oral ophicephalous pedicellaria; **C**, triphyllous pedicellaria; **D**, tridactylous pedicellaria; **E**, distal end of ophicephalous pedicellaria stalk; **F–G**, maginifications; **F**, aboral ophicephalous pedicellaria; **G**, tridentate pedicellaria. Scale bars: A–E, 100 µm; F–G: scale bars equal 50 µm.

Interambulacra—Wide, with five to six imperforate and non-crenulate primary tubercles per plate at ambitus (Figs. 3A, 4E). Smaller specimens (TD = 30 mm) bear three to four tubercles at ambitus (Figs. 3C, 4D). These tubercles with large mamelons, narrow platforms and broad areoles occupying most of plate width and deforming plate margins. Number of interambulacral plates low, usually between 14 (TD = 30 mm) and 17 (TD = 40 mm). Largest specimens reaching 89 mm TD bear up to 21 interambulacral plates. Height of plates increase quickly along aboral surface adapically (from 2 to more than 4 mm height). Above ambitus, size of tubercles reducing towards interradius and number of interambulacral primary tubercles decrease slowly towards apical system. Additional secondary tubercles developed aborally, forming second row of tubercles in adoral part of each plate (Figs. 3A, 4E). In largest specimens (TD > 45 mm), number of secondary tubercles higher, with another row of tubercles below primary tubercles (Figs. 3A, 4E). Otherwise, plates coarsely granulated in between tubercles. Epistroma well-developed, covering all plates and forming punctation or even crenulation, more easily visible in small specimens. In small specimens (TD < 20 mm), naked interradial area restricted to interradius. In larger specimens, aboral interradial region covered with both primary and secondary tubercles.

Pedicellariae—Pedicellariae of three types, ophicephalous, tridactylous and triphyllous, all abundant over test (Fig. 5). All pedicellariae are purple in adult specimens.

Ophicephalous pedicellariae (Fig. 5A, B) strongly differentiated between oral and aboral surface, more than in other *Arbacia* species. Aboral surface covered with numerous ophicephalous pedicellariae, including area surrounding apical system. Valves of ophicephalous pedicellariae low, narrow, and strongly constricted in upper part, with indented and sharply serrated blade edges (Fig. 5A). These valves very typical, being flattened or even concave at tip, with broad and deeply grooved apophyses. Numerous lateral spines in medial region of inner valve surface (Fig. 5A, F). Buccal membrane covered by second type of ophicephalous pedicellariae (Fig. 5B), with higher valves, indented blade edges, and slight serration, without spines on inner surface. Second type of ophicephalous pedicellariae also observed sparsely distributed on oral surface.

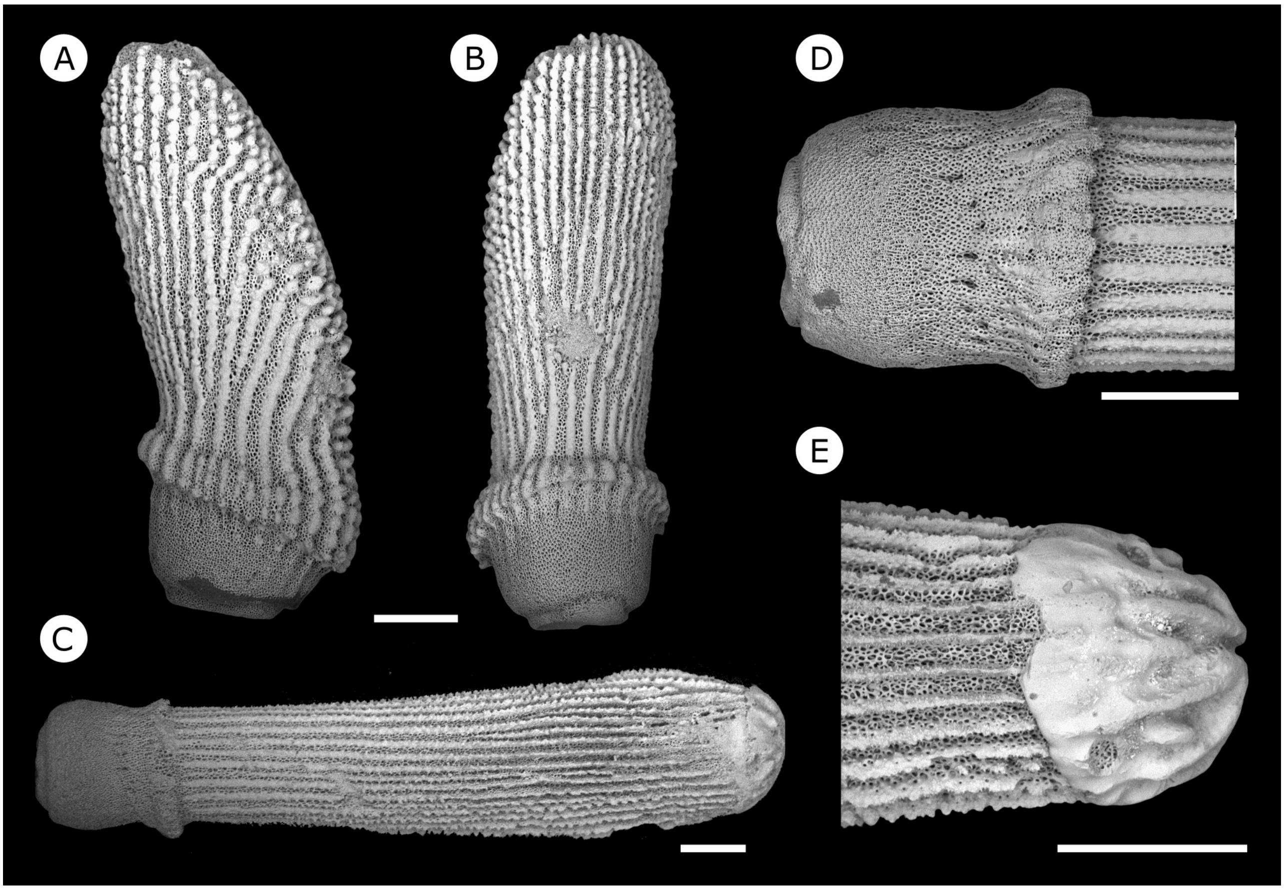

FIGURE 6. Spines of *Arbacia nigra* specimen TN_36PA; **A–B**, adapical spine; **A**, lateral view; **B**, adoral view; **C–E**, oral spine; **C**, complete oral spine; **D**, zoom on the proximal part; **E**, zoom on the distal part. Scale bars equal 500 µm.

Triphyllous pedicellariae (Fig. 5C) present but very scarce, resembling those found in other *Arbacia* species, as noted by A. Agassiz in Agassiz & Clark (1908). Suggested of absence of these made by Larraín (1975) likely erroneous, so absence of triphyllous pedicellariae not diagnostic for species, contrary to that author. Valves of triphyllous pedicellariae elongated and constricted in middle part. Blade edges finely serrated.

Tridentate pedicellariae (Fig. 5D) small, only one and half times as long as ophicephalous pedicellariae. Scarce and exclusively on oral surface, lacking on buccal membrane. Valves slender, elongated, and noticeably constricted in middle. Blade edges finely serrated. Near constriction, inner surface of valve covered by sharp, long thorns (Fig. 5D, G).

Spines—Primary spines longest at ambitus and decrease in size towards apical system. Close to apical system, spines short and large (Fig. 6A, B). Ambital and subambital spines with vestigial "enameled" tips, short relative to other *Arbacia* species (Fig. 6C, E).

Remarks

Taxonomic attribution—*Tetrapygus niger* is here re-assigned to *Arbacia*. The species was formerly assigned to *Echinus* (Linné, 1758) by Molina in 1782, under the name *Echinus niger*.

Gray (1835) established *Arbacia*, which encompassed all the species previously referred to as *Echinus* by Blainville (1825). It includes *Echinus stellatus* (= *Arbacia stellata* (Blainville, 1825)), *E. aequituberculatus* (Blainville, 1825; = *Arbacia lixula*), *E. dufresnii* (= *Arbacia dufresnii)*, *E. lixula* (= *Arbacia lixula*), as well as *E. pustulosus* (Leske, 1778) and *E. punctulatus* (Lamarck, 1816). A. Agassiz (1881) also suggested that Gray (1835) intended to include *Echinus niger* in his list of species belonging to *Arbacia*. In the same year, but one month after Gray, Desmoulin (1835) also proposed a new genus name, *Echinocidaris*, for all previously mentioned species, which led to additional confusion for decades.

Louis Agassiz (1841) decided to split *Arbacia* into three distinct genera: he restricted the name *Arbacia* to fossil species (which were presently assigned to *Cottaldia*), *Tetrapygus* to species with four anal valves (which should include all species within *Arbacia*), and *Agarites* for those species in which interambulacral areas are denuded (which might include *A. dufresnii*, *A. punctulata*, *A. stellata*, *A. spatuligera* and synonyms). This was the first use of the name *Tetrapygus*.

Troschel (1872) also split *Arbacia* into two groups: *Agarites* for those species with a denuded interambulacrum, and *Pygomma* for those species with at least one ocular plate insert. Subsequently, these two names were almost never used.

Alexander Agassiz (1863, 1881) was the first to assign *Echinus niger* sensu Molina (1782) to *Arbacia*. Later, A. Agassiz (1908) stated that *Tetrapygus* (L. Agassiz, 1841) was valid and included only this species. Since then, all subsequent authors (Clark 1909, 1925; Lessios *et al.* 2012; Mortensen 1935; Smith *et al.* 2006) followed A. Agassiz (1908) and attributed the species to *Tetrapygus*.

Mortensen (1935) considered *Tetrapygus* and *Arbacia* as "very closely related", differing only in their respective polyporous ambulacral patterns and the presence or absence of secondary tubercles, two characters that justified, for him, maintenance of two distinct taxa at the genus level.

Only Jackson (1912, 1927) assigned the species to *Arbacia* (cf. in Jackson 1927; *Tetrapygus* [= *Arbacia*] *niger*). Based on extensive observations of over 14,000 *Arbacia* individuals, Jackson (1927) argued that four characters of *Tetrapygus niger* support its classification within *Arbacia*. He stated that *Arbacia nigra* is the "most advanced species of the genus", exhibiting more progressive features compared to other species in *Arbacia*, such as a higher proportion of specimens with insert oculars, auricles of the perignathic girdle commonly forming an arch, a greater number of pore-pairs in ambulacra, and the presence of secondary spines.

Conclusion

In the present work, new molecular and morphological data support the reassignment of *Tetrapygus niger* to *Arbacia*, here designated *Arbacia nigra*. Lessios *et al.* (2012) had previously amplified the bindin gene, yielding results consistent with ours. In this study, we intended to contribute fresh insights into the genus *Arbacia*. In spite of its perceived conserved nature, the 28S gene has not yet been amplified for all studied species. Despite its lower variability, we found the 28S gene to be a reliable tool for distinguishing distinct *Arbacia* species, and a useful, conservative test for the systematic position of *Tetrapygus*. The only exception was the shared haplotype observed in *A. nigra* and *A. dufresnii,* reaffirming the very close genetic relationship between these two species. This result leads to the revision of the systematics of *Arbacia*, for which an amended diagnosis is proposed. The molecular phylogeny, based on mitochondrial (COI) and nuclear (28S) markers, consistently recovered *A. nigra* as the sister species to *A. dufresnii*. The least destabilizing taxonomic act, given this evidence, and the fact that other species in *Arbacia* occur stemward to the *A. dufresnii* plus *A. nigra* clade, is to synonymize *Tetrapygus* with *Arbacia*.

These new findings increase the number of recognized species of *Arbacia* occurring along the coasts of Chile. They also raise questions about the origin of the differentiation among the three closely related species *A. dufresnii*, *A. nigra*, and *A. spatuligera*, as well as with the fossil species *A. terraeignotae* Courville, Gonzales, Mourgues, Poulin, Saucède, 2023, and *A. larraini* Courville, Gonzales, Mourgues, Poulin, Saucède, 2023, from the upper Pliocene of northern Chile (Courville *et al*. 2023). The geographic distribution of *A. nigra* overlaps with that of *A. spatuligera*, as both species co-occur in the Warm Temperate Southeastern Pacific Province (Spalding *et al*. 2007), from 42° to 6°S latitude. However, the two species differ in their respective depth range, *A. nigra* typically being recorded in shallower waters between 0 and 10 m depth, while *A. spatuligera* is usually recorded in slightly deeper waters, between 10 and 30 m. In contrast, the distribution of *A. nigra* differs from that of its sister species, *A. dufresnii*, as *A. nigra* does not occur in the Magellanic Province.

All the data generated by this study - morphological, sequences published in GenBank and information on the coordinates of the localities - can be consulted in https://doi.org/10.5281/zenodo.10085730 (Courville *et al.* 2023b).

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