A new freshwater bivalve species of the genus *Cyclonaias* from Texas (Unionidae: Ambleminae: Quadrulini)

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ABSTRACT

Cyclonaias necki, a new species from the San Marcos River in the San Antonio/Guadalupe River Basin in Gonzalez County, Texas, USA, is here described and separated from *Cyclonaias petrina* based on shell morphology and molecular barcoding analyses.

Additional Keywords: Freshwater Bivalve, San Marcos River, Guadalupe County, Caldwell County

INTRODUCTION

On the last revision of the freshwater mussels of the United States of America and Canada, 14 *Cyclonaias* species were recognized (Williams et al., 2017), six of which occur in Texas (Howells, 2013). One of these species is the Texas Pimpleback *Cyclonaias petrina* (Gould, 1855) distributed across the Colorado and San Antonio/Guadalupe River systems. However, specimens recently collected for this study in the San Marcos River in the San Antonio/Guadalupe River Basin, revealed clear conchological and genetic differences from those in the Colorado basin, raising the question of the existence of two distinct species in south central Texas. This paper presents the description of *Cyclonaias necki* new species. **A subset of the authors of the current paper, Burlakova, Karatayev, Lopes-Lima, and Bogan, are the authors of the new species.**

MATERIALS AND METHODS

Eleven specimens, all previously identified as *Cyclonaias petrina*, were collected from the Concho River (Colorado River Basin) [4 specimens], San Saba River (Colorado River Basin) [3 specimens] and San Marcos River (San Antonio/Guadalupe River Basin) [5 specimens] (Table 1) in 2008–2011, as part of the project "State-wide Assessment of Unionid Diversity in Texas" (Federal Aid Grant No. T-43 funded by the US Fish and Wildlife Service State Wildlife Grant Program through the Texas Parks and Wildlife Department (TPWD), 2008–2012). The work was carried out with an appropriate Scientific Research

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Table 1. List of all individual specimens used for genetic analyses, catalog numbers, collection sites, and GenBank references. BSGLC (SUNY Buffalo State College Great Lakes Center); NCSM (North Carolina Museum of Natural Sciences); UF (Florida Museum of Natural History).

Species	Locality	Catalog number	River (Basin)	GenBank
Cyclonaias petrina	Paint Rock	BSGLC 1617	Concho River (Colorado)	MG969416
Cyclonaias petrina	Paint Rock	BSGLC 1619	Concho River (Colorado)	MG969417
Cyclonaias petrina	Paint Rock	BSGLC 1620	Concho River (Colorado)	MG969418
Cyclonaias petrina	San Saba	BSGLC 3254	San Saba River (Colorado)	MG969419
Cyclonaias petrina	San Saba	BSGLC 3255	San Saba River (Colorado)	MG969420
Cyclonaias petrina	San Saba	BSGLC 2157	San Saba River (Colorado)	MG969421
Cyclonaias necki	Palmetto State Park	BSGLC 1672	San Marcos River (San Antonio/Guadalupe)	MG969422
Cyclonaias necki	Luling	NCSM 65378	San Marcos River (San Antonio/Guadalupe)	MG969423
Cyclonaias necki	Luling	BSGLC 2255	San Marcos River (San Antonio/Guadalupe)	MG969424
Cyclonaias necki	Luling	BSGLC 2256	San Marcos River (San Antonio/Guadalupe)	MG969425
Cyclonaias necki	Luling	UF 441084	San Marcos River (San Antonio/Guadalupe)	KT285656

Permit SPR-0503-300 issued by the TPWD. Specimens were placed in 99% ethanol for molecular analyses. The holotype and a paratype were deposited in the North Carolina Museum of Natural Sciences (NCSM), and the remaining paratypes and other specimens in the Great Lakes Center Invertebrate Collection (BSGLC) (SUNY Buffalo State College, Buffalo, NY) (Tables 1, 2).

Sequencing, PCR Amplification, and Dataset Construction: For each sample, amplification and (bidirectional) sequencing was carried out for cytochrome c oxidase subunit I (COI) with the primers LCO_22me and HCO_700dy (Walker et al., 2006) with annealing temperature of 50°C and the remaining polymerase chain reaction (PCR) conditions as described in Froufe et al. (2014). Sequences were obtained with the BigDye sequencing protocol (Applied Biosystems 3730xl) by Macrogen Inc., Korea. Forward and reverse sequences were edited and assembled using ChromasPro 1.7.4 (Technelysium, Tewantin, Australia). All new sequences have been deposited in GenBank (Table 1). A COI dataset was then constructed with the newly sequenced individuals and one *Quadrula petrina* present in GenBank (Table 1) [San Marcos River, southwest of Luling, Caldwell/Guadlupe County Line, Texas (Pfeiffer et al., 2016)]. The dataset was aligned using the MAFFT multiple sequence alignment algorithm (Katoh and Standley, 2013) with default parameters.

Molecular-based Species Delineation Methods: Two distinct molecular methods were applied to determine the number of Molecular Operational Taxonomic Units (MOTUs). The first is distance based, i.e. the BIN system implemented in BOLD (Ratnasingham and Hebert, 2013) widely used as the standard COI barcoding method. For the BINs system, the COI dataset was analyzed with the Cluster Sequences tool implemented in BOLD 4 (http://v4.boldsystems.org) (Ratnasingham and Hebert, 2013). The second species delineation method used the 95% statistical parsimony connection limit in TCS 1.21 (Clement et al., 2000).

SYSTEMATICS

Class Bivalvia Linnaeus, 1758
Order Unionida Gray, 1854
Family Unionidae Rafinesque, 1820

Genus *Cyclonaias* Pilsbry in Ortmann and Walker, 1922

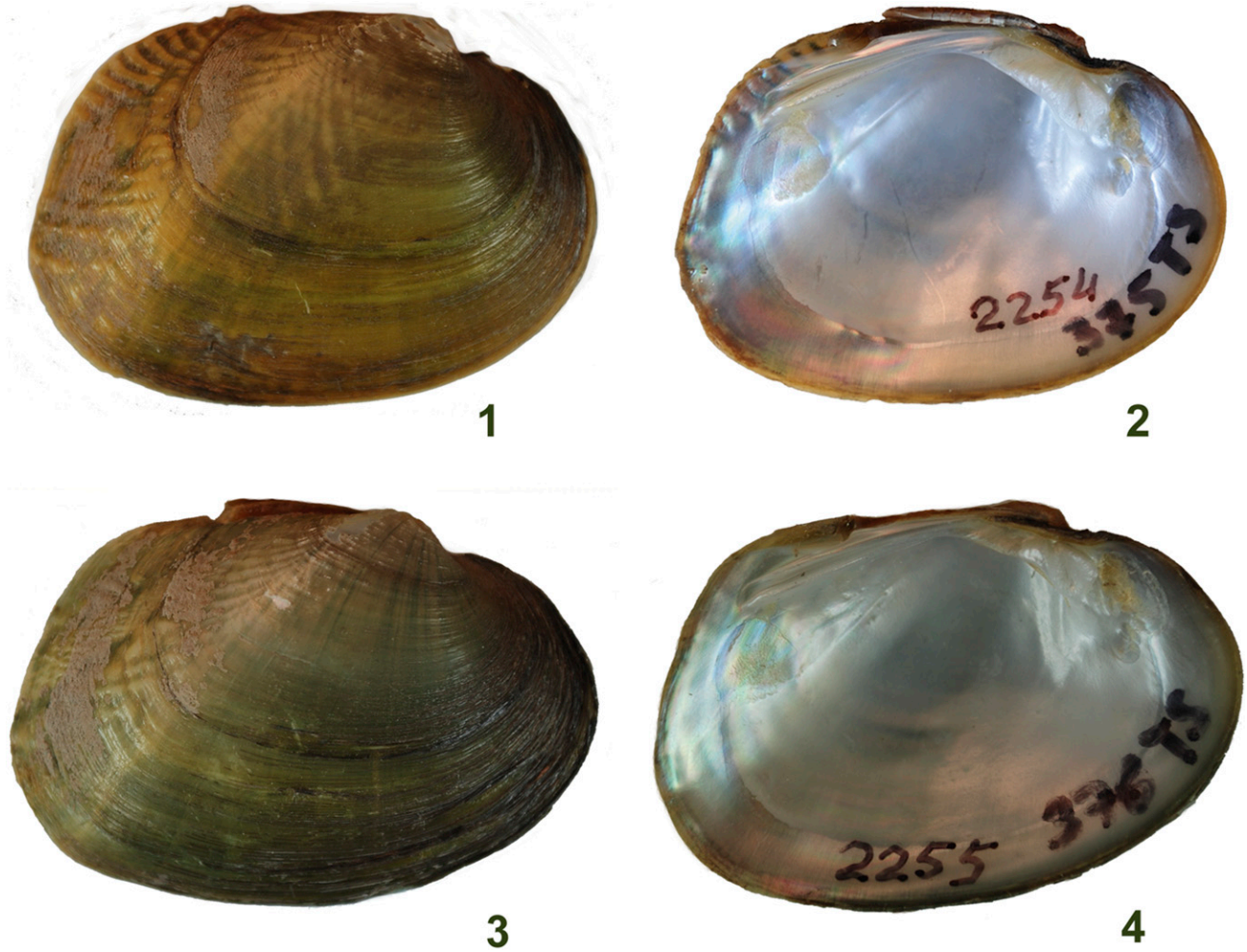
Cyclonaias necki Burlakova, Karatayev, Lopes-Lima, and Bogan new species

Common Name: Guadalupe Orb
(Figures 1–4)

Comparative Diagnosis: Younger shells of *Cyclonaias necki* new species are diagnosed by the distinct corrugations

Table 2. Measurements of *Cyclonaias necki* new species type specimens.

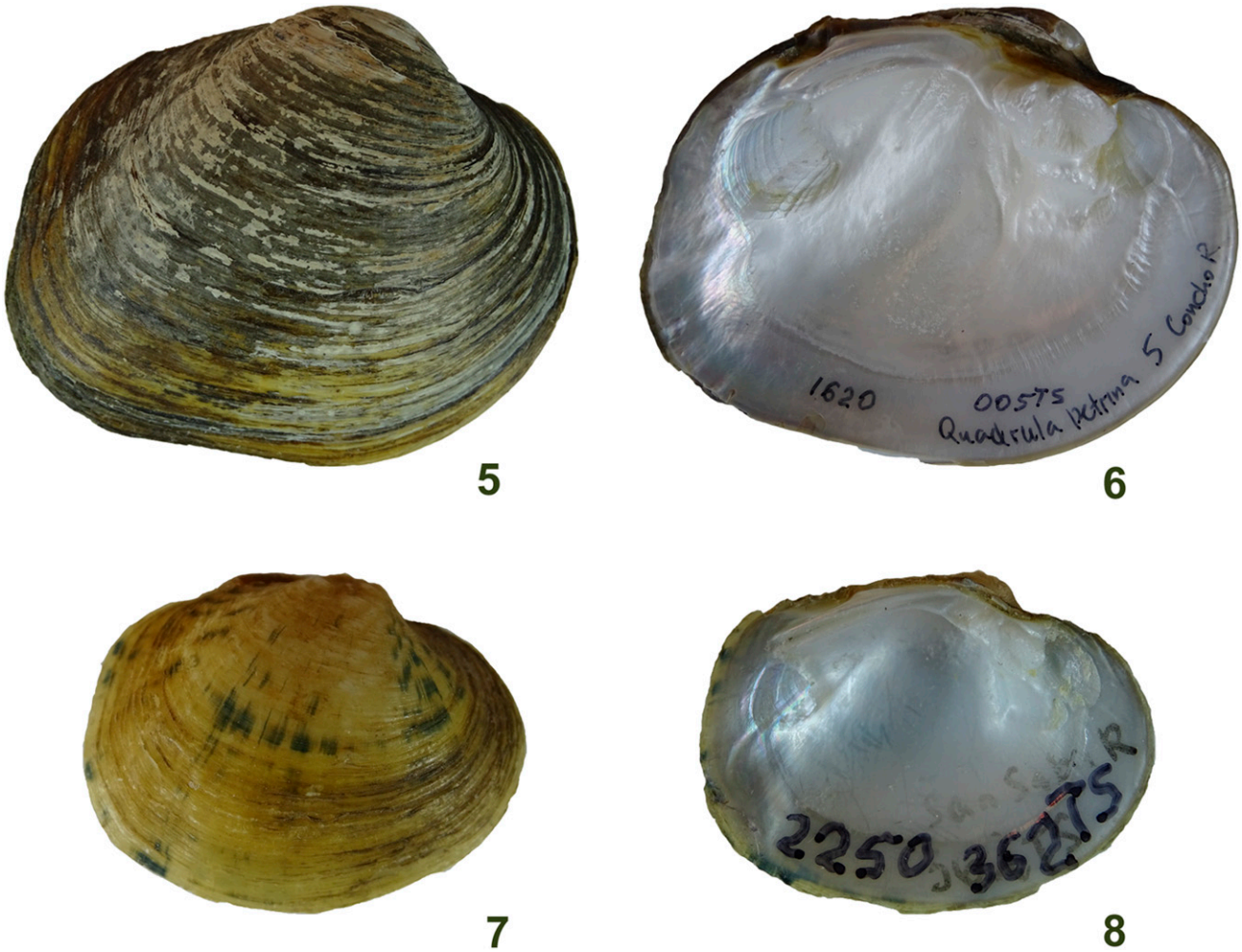
Type	Catalog number	Shell length mm	Shell height mm	Status
Holotype	NCSM 65378	42	30	Live
Paratype	NCSM 65379	60	42	Live
Paratype	BSGLC 2251	63	42	Live
Paratype	BSGLC 2252	58	38	Live
Paratype	BSGLC 2253	54	37	Live
Paratype	BSGLC 2255	43	30	Live
Paratype	BSGLC 2256	45	32	Live
Paratype	BSGLC 2257	40	29	Live
Paratype	BSGLC 2258	47	33	Fresh dead
Paratype	BSGLC 2259	40	28	Fresh dead
Paratype	BSGLC 2260	36	27	Fresh dead



Figures 1–4. *Cyclonaias necki* new species. **1–2.** Holotype NCSM 65378, shell length 42 mm. **1.** Outside of right valve. **2.** Inside of left valve. **3–4.** Paratype BSGLC 2255, shell length 43 mm. **3.** Outside of right valve. **4.** Inside of left valve.

across the posterior slope, extending onto the disk of the shell from the posterior ridge, and lack of pustules (Figures 1–4). *Cyclonaias petrina* (Figures 5–8) has weaker or missing corrugations, typically lack the corrugations on the disk of the shell, and may have occasional pustules. *Cyclonaias petrina* has a thick, inflated shell with a rounded shell disk and round shell shape with an indistinct posterior ridge. Shells of *Cyclonaias necki* new species are thinner, more compressed and rectangular, and the posterior ridge is more distinct and prominent. The umbo on shells of *C. petrina* appears broader and more inflated, while in *C. necki* new species, it is more compressed. Pseudocardinal teeth in *C. petrina* are larger and thicker than in *C. necki* new species. The interdentum of both species are about the same size in relation to shell length. Lateral teeth are short in both species. The umbo cavity of *C. petrina* is deep and open, but appears slightly more compressed in *C. necki* new species. Both species are closely related to *Cyclonaias nodulata* (Rafinesque, 1820), which is very inflated, thick shelled, and usually characterized by two rows of pustules down the disk of the shell, but lacking a sulcus between the rows as seen in *Quadrula quadrula* (Rafinesque, 1820).

Description: Shell length reaches about 63 mm, shell height about 42 mm. Shell shape subquadrate to suboval, shell moderately inflated and relatively thin. Anterior shell margin broadly rounded, dorsal shell margin straight to slightly convex, ventral shell margin slightly convex, posterior shell margin obliquely truncate. Posterior ridge rather well-defined becoming rounded with age. Some specimens have a secondary ridge between posterior ridge and posterior shell margin. Posterior slope covered with fine corrugations running from posterior ridge to posterior shell margin. Umbo area full and well developed, rising above hinge line. Umbo sculpture begins as a series of bars, becomes bumps at either end of a bar, diverging to small bumps down umbo. Periostracum yellowish green to green becoming brown with age, often with fine green rays becoming obscured with age. Shell surface smooth with no pustules, some younger specimens with corrugations extending from posterior ridge anterior ventrally,



Figures 5–8. *Cyclonaias petrina*. **5–6.** BSGLC 1620, shell length 88 mm, Concho River, Concho County, Texas 31.51982N, 099.94063W. **5.** Outside of right valve. **6.** Inside of left valve. **7–8.** BSGLC 2250, San Saba River, San Saba County, Texas, 31.21064N, 098.74124W; shell length 26 mm. **7.** Outside of right valve. **8.** Inside of left valve.

disappearing by central area of shell disk. Sculpture becoming obscured or eroded in older specimens. Single large pseudocardinal tooth in right valve with a vestigial tooth anterior to main tooth, two large sculptured teeth in left valve. Interdentum is well developed in both valves. Lateral tooth single and straight in right valve with two well defined teeth in left valve. Anterior adductor muscle scar deep and smooth, pedal protractor muscle scar deep, separate from, and located posterior and slightly ventral to anterior adductor muscle scar. Anterior pedal retractor muscle scars are deep pits at base of pseudocardinal teeth and almost merge with anterior adductor muscle scar. Posterior adductor muscle scar well defined, but shallow, posterior pedal retractor muscle scar dorsal to posterior adductor muscle scar and merges with posterior adductor muscle scar. Pallial line well defined anteriorly and becoming fainter posteriorly. Umbo cavity deep and open. Nacre color white and iridescent posteriorly.

Type Material: Holotype: NCSM 65378 [ex BSGLC 2254]; **Paratypes:** BSGLC 2251, 2252, 2253, 2255, 2256, 2259, 2260; NCSM 65379 [each lot is a single specimen] (Table 2). All from type locality, collected by Lyubov E. Burlakova, Alexander Y. Karatayev, Vadim A. Karatayev, on July 12, 2011.

Type Locality: San Marcos River, between US90 and SR80, southwest of Luling, Caldwell/Guadalupe counties (the river is the border), Latitude 29.67078 N, Longitude 097.69561 W.

Comparison with Similar Species: *Cyclonaias necki* new species was reported by Strecker (1931) as a small variety, but not separate from *C. petrina*. *Cyclonaias necki* new species was noted by Howells (2013) as "the ecophenotypes in the Guadalupe-San Antonio drainage are often somewhat more elongate, thinner shelled, and with less robust hinge teeth." *Cyclonaias aurea* (Lea, 1859) resembles *C. necki* new species but lacks the corrugations on the posterior slope and the disk of the shell. *Cyclonaias nodulata* (Rafinesque, 1820) is more inflated, thicker

shelled than *C. necki* and is marked by two variable rows of pustules or knobs down the disk of the shell, but lacking a sulcus found in *Quadrula quadrula* and *Q. apiculata* (Say, 1829) and the numerous pustules of these two species. *Cyclonaias necki* new species resembles some *Fusconaia* species, but these species lack the corrugations on the posterior slope.

Molecular Analyses: The COI alignment was 596 base pairs (bp) long and included 7 haplotypes, 4 from the San Antonio/Guadalupe River drainage and 3 from the Colorado River Basin. Two MOTUs were identified by both species delineation methods: one corresponding to the specimens of *Cyclonaias petrina* from the Colorado basin and another corresponding to the specimens of *Cyclonaias necki* new species from the San Marcos River. The interspecific COI divergence (3.9%, uncorrected *p*-distance) is within the usual range for the Unionidae (Prié and Puillandre, 2014; Lopes-Lima et al., 2017). The intraspecific COI divergence within each MOTU was <1%.

Distribution: *Cyclonaias petrina* was recognized from the Colorado River Basin and "A small variety is found in the Guadalupe River in Comal County" was reported by Strecker (1931). It was subsequently reported from the Guadalupe and Colorado River basins and from the Llano, San Saba, and Pedernales rivers (Howells et al., 1996), and recently from the Concho, Guadalupe, San Saba, and San Marcos rivers (Burlakova et al., 2011; Burlakova and Karatayev, unpublished data). The range was listed as "endemic to the Colorado and Guadalupe-San Antonio systems of Central Texas" (Howells, 2013). *Cyclonaias petrina* was recognized by Williams et al. (2017). Here, we have separated *C. necki* new species from *C. petrina* and recognize *C. petrina* as restricted to the Colorado River Basin with *C. necki* new species endemic to the Guadalupe-San Antonio River Basin of Central Texas.

Habitat and Biology: Host fish and the period of gravidity are unknown. *Cyclonaias necki* new species was found living in a small river in flowing water with a sand and gravel substrate, mostly in water less than 2 m.

Conservation Status: *Cyclonaias petrina* was considered threatened (Williams et al., 1993). This new species has been split from *Cyclonaias petrina* which is listed by Texas Parks and Wildlife Department as legally threatened (Howells, 2013). The US Fish and Wildlife Service (USFWS) has been petitioned to examine *C. petrina* for federal listing as threatened or endangered (USFWS, 2018). Their evaluation reported *C. petrina* as having occurred historically in 38 counties, but was listed only from 16 counties today. By recognizing *C. necki* new species from the San Antonio/Guadalupe River Basin, this restricts the modern distribution of *C. petrina* to eight counties in the Colorado River Basin and the range of *C. necki* new species to eight counties. This defines the modern range of *C. petrina* to half of the USFWS listed counties (USFWS, 2018). Considering the restricted range, we would suggest *C. necki* new species and *C. petrina* warrant listing as endangered.

Etymology: We take this opportunity to name the new species, recognizing his work on freshwater mussels of Texas, after Raymond W. Neck, a pleasant and helpful friend (1946–2017).

Comparative Material Examined: Shells of *Cyclonaias petrina* examined for comparison included BSGLC 1620, 2128, 2133, 2156, and NCSM 33710 and 33713.

ACKNOWLEDGMENTS

Mrs. Cynthia M. Bogan and Ms. Jamie M. Smith have reviewed this manuscript and provided helpful comments. Ms. Smith is thanked for removing the background of the eight figures used here.

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