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Age, Growth and Reproduction of Western North Atlantic Butterfly Rays (Myliobatiformes: Gymnuridae), with the Description of Two New Species

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http://dx.doi.org/doi:10.21220/V58T7S

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Age, Growth and Reproduction of Western North Atlantic Butterfly Rays (Myliobatiformes: Gymnuridae), with the Description of Two New Species

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A Dissertation

Presented to

The Faculty of the School of Marine Science

The College of William and Mary in Virginia

In Partial Fulfillment

of the Requirements for the Degree of

Doctor of Philosophy

---

by

Kristene Teal Parsons

August 2017

APPROVAL PAGE

This dissertation is submitted in partial fulfillment of

the requirements for the degree of

Doctor of Philosophy

Kristene Teal Parsons

Approved by the Committee, May 2017

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DEDICATION

To my parents, D.J. Parsons, P.J. Parsons, and T.A. Crowley, for their constant encouragement and decades of nurturing my interests in elasmobranch fishes, and to S.H. Gruber and J.F. Morrissey for their mentorship, guidance, and support of my academic pursuits.

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ACKNOWLEDGEMENTS

The completion of this dissertation and my doctoral degree is an achievement that I share with the numerous people who contributed their time and effort to support my research. I am thankful for each of my committee members, who were instrumental in the development of my research through their guidance and participation in various studies. R. Latour provided academic support, and spent many hours sharing his mathematical and statistical expertise to help develop my own quantitative skill set. J. McDowell was a constant source of encouragement and collaboration, and her contributions to the molecular analyses directly influenced the outcome and impact of my research. I am indebeted to her genuine interest in my work and her friendship. It has been an honor to learn from and work beside E. Hilton. Through his professorship, mentorship, and innate obsession with the natural world, Eric has inspired and motivated my academic growth beyond ichthyology, and I am grateful for his example and many contributions to this dissertation. W. Vogelbein played a key role in the final stretch of my academic tenure, and I thank him for our discussions and his keen interest in my work. Finally, I thank C. Cotton, who welcomed me into the Fisheries Department and shark program years ago, and has transitioned from colleague to mentor and dear friend throughout this process. Chip's knowledge of elasmobranch life history was fundamental to the foundation of my work, and he instilled in me the value of scientific skepticism, for indeed "*Science is the belief in the ignorance of experts"* - Richard Feynman. I am grateful for his intellectual and practical contributions to this dissertation, his playlist aboard the R/V *Bay Eagle*, and his humor and encouragement to persevere.

I extend my deepest gratitude to J. Musick and Captain D. Ward, two gentlemen who had immense influence on my VIMS education. Jack unofficially served on my committee through his thoughtful inquiries and discussions, and his matter-of-fact commentary and wit. Despite his emeritus status, Jack continues to engage the studentship, inspiring while simultaneously challenging each of us to keep learning – he remains a true gem of the VIMS community. I am honored to have contributed to Jack's legacy, and it was Durand's acceptance aboard the R/V *Bay Eagle* that enabled me to do so. Durand welcomed me onto the shark survey, taught me invaluable lessons in life, longlining, and everything in between, and kept me anchored when the winds caught my sail. Indeed, those long, hot days at sea smelling of bunker and diesel in Durand's company were the most gratifying of my 9 years at VIMS.

There are many others who aided in my academic and professional growth at VIMS. I thank J. Graves, J. Olney, M. Fabrizio, I. Anderson, C. Forrester, G. Tisdale, and F. Powell for their administrative assistance and guidance. I am also grateful to the VIMS office of Academic Studies (L. Schaffner, C. Cake, J. Hay), the VIMS Foundation, and the Graduate Student Association for funding my academic and research activities. When needed most, L. Lawson, P. Nichols, D. Gonzales, D. Sennett, R. Billups, P. Crewe, P. Richardson, M. Seebo, and T. Tuckey were always there to remind me not to take anything too seriously, and I thank them for their camaraderie.

The earliest roadmap for this dissertation was conceptualized aboard the F/V *Darana R* while working with the VIMS Multispecies Research Group in 2012. Through conversations with J. Gartland and J. Rhule over our large catches of skates and rays, it became clear that very little was known about Mid-Atlantic batoid populations. I am thankful to them both for their insight and logistical and moral support throughout the years that followed, and I also appreciate the efforts of B. Rhule and crew. The past and present staff of the Multispecies Research Group were integral to this dissertation through their collection and contribution of specimens and data, assistance in the laboratory, participation in analysis and manuscript preparation, and general tolerance of my excitement for elasmobranchs. I especially appreciate C. Bonzek, J. Gregg, D. Gauthier, G. Mears, C. Ward, K. Spanik, D. Gregg, and M. Chattin for their contributions and many years of friendship. The staff of the Juvenile Fish and Blue Crab Survey also contributed valuable specimens to my research, thanks to the extra efforts of T. Tuckey, W. Lowery, E. Loose, and V. Hogge. Interns J. White, M. Seitz, and K. Aguiar Gabaldo deserve recognition for dedicating their time to participate in my research, and I thank them for reminding me why I chose this field in the first place. Credit for work in the molecular laboratory is due to H. Brightman, who graciously took on this project and became a close friend and source of support in the process.

There are several external agencies, institutions, and laboratories that provided specimens and data critical to this dissertation. I thank the staff, captains and crew of the Northeast Fisheries Science Center Multispecies Bottom Trawl Survey, the Southeast Area Monitoring & Assessment Program (SEAMAP) Bottom Trawl Survey and Sea Turtle Trawl Survey (South Carolina Department of Natural Resources, Marine Resources Research Institute), the Cooperative Atlantic States Shark Pupping & Nursery (COASTSPAN) Bottom Longline Survey (University of North Florida, Shark Biology Program), the Fisheries Independent Monitoring Program Bottom Trawl Survey (Florida Fish and Wildlife Research Institute), the Gulf of Mexico Shark Pupping & Nursery (GULFSPAN) Bottom Longline & Gillnet Survey (Florida State University Coastal and Marine Laboratory), the Gulf Specimen Marine Laboratory, Dauphin Island Sea Lab Discovery Hall Program (University of South Alabama), and the Coastal Fisheries Division Bottom Trawl Survey (Texas Parks & Wildlife Department). Special thanks are extended to collaborators J. Galbraith, P. Chase, M. Stratton, M. Kolmann, A. Kroetz, G. Graeber, M. Drymon, T. DiGirolamo, R. Brodie, J. Gelsleichter, M. McCallister, L. Parker, J. Dingle, J. Schwenter, M. Arendt, F. Hernandez, and J. and C. Rudloe. Thanks to the University of Texas High-Resolution CT Facility, particularly J. Maisano for CT scanning and reconstruction, and her contributions to the age and growth study and manuscript.

I am incredibly grateful for the assistance of T. Willems, Captain S. Hall, K. Wan, V. Rozy, A. Gangadin, M. Kolmann, and T. Nalovic, who collectively ensured a successful expedition to Suriname. I also thank the Anton de Kom University of Suriname, the National Zoological Collection of Suriname, and The Ministry of Agriculture, Animal Husbandry and Fisheries for logistical support, laboratory space, and collection permission. The following museum collections, staff, and curators made significant contributions to the taxonomy study, and were gracious hosts: J. Williams, K. Murphy, S. Raredon, and S. Smith and the National Museum of Natural History Museum Support Center, Smithsonian Institution, Washington, D.C.; K. Hartel, A. Williston, and the Harvard University Museum of Comparative Zoology, Cambridge, MA; S. Mochel, K. Swagel, C. McMahon, and the Field Museum, Chicago, IL; R. Robins, G. Burgess, D. Boyd, Z. Randall, and the Florida Museum of Natural History, Gainesville, FL; N. Schnell, Z. Gabsi, and the Muséum National d'Histoire Naturelle, Paris, France; S. Huber and the Nunnally Ichthyology Collection, Gloucester Point, VA. I am also grateful to the Museum of Comparative Zoology for providing travel support for this research through the Ernst Mayr Travel Grant in Animal Systematics.

My journey to VIMS was most strongy influenced by my closest mentors, advisers, colleagues, and friends S. and M. Gruber, J. Morrissey, D. Grubbs, S. Newman, and M. Calosso. I

am humbled by the opportunities and experiences that each provided me to help steer my course, and I thank them for their guidance, advocacy, and unwavering support that culminated in this degree. I also thank M. Kolmann and L. Parenti for their counsel and discussions that were pivotal to the development of this dissertation.

I owe my gratitude to my VIMS family for building a support network that never failed me. I am particularly fortunate to have shared my time here with K. Sobocinski and M. Nelson, K. Hill Spanik and K. Spanik, G. Ralph, G. Saluta, T. Clardy, M. Whalen, A. Then, A. Deary, J. and W. Hudson, P. and P. McGrath, A. Wozniak, T. Nalovik, K. Omori, L. Ailloud, C. Martinez, K. McMillan and M. Stratton, B. Conroy, C. Peterson, D. Gauthier, M. Chattin, K. Bemis, and D. Vaz.

I especially thank my parents D. Parsons, P. Parsons, and T. Crowley, in addition to my extended families, for believing in me and never questionning my aspirations to study elasmobranchs. I am forever indebted to them for sacrfices they made to help me along the way, and for their constant love and push to keep going. Finally, I thank C. Sweetman, who has been the rock keeping my head above water when tides were high. CJ's contributions to the completion of this degree and dissertation over the years were many, and my survival in this program is a testament to his support, encouragement, and companionship, for which I am eternally grateful.

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SP contributed most to differences between geographic regions (a) and individual variability (b). The proportion of variation explained without LNC was 73% and 27% for CA1 and CA2, respectively, and significant regional separation of specimens was retained by the remaining nine characters (c, d)

Figure 4. Canonical Correlation Analysis plots of 10 morphometric characters (WIO, WIS, LSV, DG5, DG1, SP, LNC, WIN, WNC, WM) of juvenile and adult female specimens of the *Gymnura* complex from the western North Atlantic (ATL), Gulf of Mexico (GOM), and Suriname (SUR – including Venezuela and French Guiana). The first canonical axis (CA1) and CA2 accounted for 78% and 22% of the variation explained, respectively, and LNC and SP contributed most to differences between geographic regions (a) and individual variability (b). The proportion of variation explained without LNC was 71% and 29% for CA1 and CA2, respectively, and significant regional separation of specimens was retained by the remaining nine characters (c, d)

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AUTHOR'S NOTE

This dissertation comprises three chapters, each written in manuscript format for scientific publication. Journal guidelines for manuscript submission were followed in the formatting of chapters. At the time of writing, citations for individual chapters are:

CHAPTER 2

Parsons, K.T., Maisano, J.A., Gregg, J.W., Cotton, C.F. and R.J. Latour. In Review. Age and growth assessment of western North Atlantic spiny butterfly ray *Gymnura altavela* (L. 1758) using computed tomography of vertebral centra. *Environmental Biology of Fishes*.

CHAPTER 3

Parsons, K.T., Cotton, C.F. and R.J. Latour. In Prep. Aspects of reproductive biology in the spiny butterfly ray (*Gymnura altavela*) and smooth butterfly ray (*Gymnura micrura*) from the western North Atlantic and Gulf of Mexico. *Journal of Fish Biology*.

CHAPTER 4

Parsons, K.T., Hilton, E.J., McDowell, J.R., Brightman, H.L. and R.J. Latour. In Review. Morphological and genetic analyses of *Gymnura micrura* (Myliobatiformes: Gymnuridae) from the western North Atlantic Ocean reveal two new species of Butterfly Ray. *Journal of the Ocean Science Foundation*.

DISSERTATION ABSTRACT

Batoid fishes are among the most threatened and least understood chondrichthyan species worldwide due to their large body size, conservative life-history characteristics, and predominantly coastal distributions where fishing and habitat degradation threaten the stability of populations. A lack of empirical life history data is widespread across batoid taxa — nearly half of all species are considered data deficient, thus hindering species assessments and the development of effective management strategies. Furthermore, many batoid taxa are in need of taxonomic re-examination. Increasing our understanding of life history traits that determine population productivity, such as age and size at maturity, growth rate, and fecundity is prerequisite to examining the potential for populations to increase or stabilize in response to fishing mortality.

The Butterfly Rays (Myliobatiformes: Gymnuridae) are comprised of 10 globally distributed species that inhabit shallow coastal regions and are commonly caught in benthic fishing gears targeting commercially valuable species. Two species are recognized in the western Atlantic: the Spiny Butterfly ray, *Gymnura altavela* (Linnaeus 1758), and the Smooth Butterfly Ray, *G. micrura* (Bloch & Schneider 1801). Previous life history studies on U.S. Butterfly Rays were often spatially and temporally limited, which may bias conclusions due to underrepresentation of some life stages, and lead to inaccurate biological characterizations. Furthermore, sexual dimorphism and ontogenetic variability in body shape, and inter- and intraspecific inconsistencies in taxonomic characters (e.g., disk coloration, tail banding patterns) have contributed to substantial taxonomic confusion in the Gymnuridae.

To address knowledge gaps in the life history and taxonomy of western Atlantic Butterfly Rays, this dissertation describes the age and growth of *G. altavela*, the reproductive biology of *G. altavela* and *G. micrura*, and the taxonomic status of *G. micrura*. The largest male and female *G. altavela* were estimated to be 11 and 18 yrs old, respectively. Disk width at maturity was 1278 mm and 946 mm for male and female *G. altavela*, respectively, and was significantly greater in Atlantic *G. micrura* (male: 390 mm; female: 551 mm) than Gulf of Mexico *G. micrura* (male: 298 mm; female: 448 mm). Maximum fecundity was seven in *G. altavela*, and ranged from six to 12 in *G. micrura* from the Atlantic and Gulf of Mexico, respectively. Based on geographical variation in life history parameters, morphology, and genetics, a re-description and proposed neotype for *G. micrura* is presented, and two new species and holotypes are described from the Atlantic (*Gymnura* n. sp. A) and Gulf of Mexico (*Gymnura* n. sp. B). In U.S. waters, *Gymnura* n. sp. A may be more vulnerable than *Gymnura* n. sp. B to indirect fishing mortality due to its larger size, potential later age at sexual maturity, and lower fecundity, since the probability of an individual encountering fishing gear before successfully reproducing is likely greater. This disseratation provides empirical support for the conservation and sustainable management of Atlantic Butterfly Rays. Careful consideration of species-specific taxonomy and biology is required to accurately assess the vulnerability of contemporary populations to extinction risk, and to document and maintain the true biodiversity of this taxon.

Age, Growth and Reproduction of Western North Atlantic Butterfly Rays (Myliobatiformes: Gymnuridae), with the Description of Two New Species

INTRODUCTION

Background

The chondrichthyan fishes comprise an estimated 1250 living species of sharks, skates, rays and their allies whose ancestors originated over 400 million years ago (Bräutigam et al., 2015). The success of these cartilaginous fishes is demonstrated by the variety of aquatic ecosystems and niches in which they are foundhave adapted, from the coastal, pelagic, and deep realms of all oceans to estuaries, freshwater rivers, and inland lakes. Batoid fishes (skates, rays, guitarfishes, wedgefishes, and sawfishes) represent more than half of all described chondrichthyan species, yet remain poorly understood and include some of the world's most threatened vertebrate species despite their evolutionary success (Bräutigam et al., 2015).

Relative to teleostean fishes, batoid fishes generally grow more slowly, require several years to reach sexual maturity, and produce fewer offspring throughout their lifetime—traits that result in increased vulnerability of populations to depletion from overexploitation, bycatch, the degradation and loss of habitat, and climate change (Brander 1981; Dulvy & Reynolds, 2002; Simpfendorfer et al., 2011; Dulvy et al., 2014). Consequently, reported increases in global batoid landings have generated management and conservation concerns; thus improved monitoring of populations and a better understanding of the life history of taxa worldwide is urgently required (Sulikowski et al., 2005; Kyne et al., 2012; Mandelman et al. 2012; Dulvy et al, 2014).

The International Union for the Conservation of Nature (IUCN) Red List of Threatened Species found that nearly 20% of all batoid fishes are threatened with extinction (www.iucnredlist.org). Assessment of the extinction risk for these species is hindered by inadequate information, given that more than 45% of batoids that are considered 'Data Deficient'. This deficiency presents major challenges for the development of effective management strategies (Dulvy et al., 2014). However, successful management and conservation of this group requires improved understanding of species-specific life history strategies, for which taxonomic clarity is a fundamental requirement. For many taxa, early species descriptions were often brief and did not fully account for ontogenetic, sexually dimorphic, or individual morphological variability, resulting in taxonomic confusion, misidentifications, and uncertainty in the status of many species. Collectively, the lack of knowledge for batoid fishes has implications that extend beyond the conservation concerns for this group. For example, in their ecological role as important mesopredators and prey that link upper and lower trophic levels, many batoid fishes contribute to the structure and dynamics of coastal ecosystems (Heithaus et al. 2010; Bornatowski et al. 2014), suggesting that stability and productivity of co-occurring ecologically and economically valuable fauna may be impacted by perturbations to batoid populations (Heithaus et al., 2008; Stevens et al., 2000). Improved understanding of the taxonomy, life history, and ecological role of batoid fishes is therefore essential for the assessment, management, and conservation of species facing increasing environmental and anthropogenic pressures that can influence the biodiversity and stability of aquatic ecosystems. To address the need for increased knowledge of batoid fishes, this dissertation presents an investigation into the life history and taxonomy of two western North Atlantic Butterfly Rays (Myliobatiformes: Gymnuridae, van Hasselt 1823).

The Gymnuridae are globally distributed in warm temperate and tropical seas where they inhabit shallow coastal regions dominated by sandy and muddy substrates (McEachran &

Capapé, 1984; McEachran & Séret, 1990; Murdy et al., 2013; Last & Stevens, 2016). The family comprises one genus (*Gymnura*) containing 10 recognized species, and the Indo-West Pacific is the most species-rich region of its distribution (Jacobsen & Bennett, 2009; Last et al., 2016). Members of the Gymnuridae are distinguished from other rays by a rhomboid and highly dorso-ventrally compressed body shape, in which the width of the disk is approximately twice the disk length, and by a short and slender tail that often has light and dark crossbars (Compagno & Last, 1999; Last & Stevens, 2009). Although they are commonly described as benthic species, the Butterfly Rays are unique among other Myliobatiformes in their form of locomotion, and are capable of both undulatory and oscillatory swimming modes, typically linked to benthic and pelagic habitat use, respectively (Rosenberger, 2001). Diet studies have also revealed that the *Gymnura* are tertiary, piscivorous, and occupy one of the highest trophic levels (i.e. 4.24) of all batoid fishes examined (Raje 2003; Bizzarro 2005; Jacobsen & Bennett, 2013; Yokota et al., 2013).

In the western North Atlantic, two species are recognized: the Spiny Butterfly Ray, *Gymnura altavela* (Linnaeus 1758), and the Smooth Butterfly Ray, *G. micrura* (Bloch & Schneider 1801). Both species are distributed from the U.S. Mid-Atlantic coast and Gulf of Mexico to the coast of Brazil (Robins & Ray, 1986; McEachran & de Carvalho, 2002; Last et al., 2016), and are also reported from the western coast of Africa in the eastern Atlantic, in addition to the Mediterranean Sea where *G. altavela* can be found (McEachran & Séret, 1990; Ebert & Stehmann, 2013). *Gymnura altavela* is easily distinguished from *G. micrura* by a significantly larger adult body size, the presence of one or more tail spines, and a tentacle-like lobe on the margin of each spiracle (Last et al., 2016). Biological information on *G. altavela* and *G. micrura*, including diet, taxonomy, and reproduction has been reported in several studies (e.g., Daiber & Booth, 1960; Capape *et al*., 1992; Yokota *et al*., 2012; Alkusairy *et al*., 2014). Despite reported disk widths exceeding 2 m (Bini 1967; Schwartz 1984; Bigelow and Schroeder 1953), there is no information on age and growth for any species of Butterfly Ray, presumably due to difficulties

interpreting growth bands in their relatively small and poorly mineralized vertebral centra. Furthermore, previous investigations into other aspects of the life history of these gymnurid species were often spatially and temporally limited, which may bias conclusions due to underrepresentation of some life stages, and lead to inaccurate biological characterization and taxonomic confusion. Uncertainty in the taxonomic status of gymnurids remains problematic due to the lack of type material for some species. Consequently, taxonomic revision of the Gymnuridae and re-descriptions of most taxa are needed (Muktha et al., 2016; Jacobsen & Bennett, 2009; Smith et al., 2009).

Gymnurids are incidentally caught in trawls and other benthic fishing gears targeting demersal species in U.S. waters, and high catches are common in some coastal and estuarine regions (Shepherd & Myers, 2005; Grubbs & Ha, 2006; K. Parsons, pers. obs.). In the western North Atlantic, Butterfly Rays are not considered species of commercial value, and therefore their populations are not managed or directly monitored. Both *G. altavela* and *G. micrura* are considered species of Least Concern in U.S. waters by the IUCN, although widespread population declines of *G. altavela* in other regions have resulted in a global status of 'Vulnerable', and the species is 'Critically Endangered' in the Mediterranean (Walls *et al*., 2016) and the Southwest Atlantic off Brazil (Vooren *et al*., 2007). All *G. micrura* populations are considered 'Data Deficient', and accurate assessments of catches throughout the geographical range of the species are needed (Grubbs & Ha, 2006).

Dissertation Rationale and Summary

The Mid-Atlantic Bight encompasses coastal areas that are well sampled by VIMS fishery-independent survey programs, and catch data imply the potential importance of this area to a large community of at least 13 batoid species, one quarter of which are classified as 'Data

Deficient' by the IUCN. Survey programs provide a valuable platform for the collection of species-specific data on the taxonomy, life history, abundance, and distribution of batoid species. Monitoring and assessment of the majority of batoid species is, however, not a priority for most programs, resulting in poor or non-existent data on batoid populations relative to other invertebrates, teleosteans, chondrichthyans, and sea turtles. To address the clear need for improved data collection and analysis in order to better understand the status and role of batoid species in coastal ecosystems of the western North Atlantic, I focused my dissertation research on two sympatric species of *Gymnura* that are common in the Mid-Atlantic Bight: *Gymnura altavela* and *G. micrura*. Given that the ranges of distribution for both species extend far beyond the Mid-Atlantic Bight, it was essential to collaborate with 11 survey programs from Massachusetts to Texas to access specimens representative of the populations in U.S. waters. More than 650 specimens of *Gymnura* were collected through this effort with the primary goal to identify key life history parameters that can be used to assess the status and vulnerability of U.S. populations. In Chapters 1 and 2, I address knowledge gaps in the biology and life history of *Gymnura* with the following objectives: (1) determine the age of *G. altavela* from vertebrae using High Resolution X-ray Computed Tomography; (2) describe growth patterns and estimate key growth parameters for this species; and (3) describe sexual dimorphism and variation in the size at reproductive maturity, reproductive anatomy, periodicity, and fecundity of *G. altavela* and *G. micrura*. Latitudinal and regional variation in the life history of *G. micrura* revealed in Chapter 2 raised concerns for the status of the species, thus providing the foundation and motivation for Chapter 3, with the aim to clarify the taxonomic uncertainty of *G. micrura* based on morphological, molecular, and life history data. To achieve this objective, data from fresh specimens collected for Chapter 2 were augmented with data from nearly 300 preserved specimens of *Gymnura* that represented nine of the 10 valid species from 28 countries held in the collections of the National

Museum of Natural History, Smithsonian Institution, Washington, D.C. (USNM), the Harvard University Museum of Comparative Zoology, Cambridge, MA (MCZ), the Field Museum, Chicago, IL (FMNH), the Florida Museum of Natural History, Gainesville, FL (FLMNH), and the Muséum National d'Histoire Naturelle, Paris, France (MNHN). Taxonomic evaluation of *G. micura* was also dependent on morphometric and molecular comparisons between U.S. specimens and those from the type locality (i.e. Suriname) for the species, since a holotype was not documented when the species was originally described by Bloch & Schneider in 1801. Accordingly, I embarked on an expedition to the northern coast of South America to acquire specimens from Suriname. Data from the type-locality specimens proved to be invaluable, necessitating the transformation of Chapter 3 from a taxonomic evaluation of *G. micrura* to a re-description of the species from Suriname, and the description of two new species of Butterfly Ray in the western Atlantic.

In summary, the impetus driving my research questions was the paucity of the most basic biological information for two common, but poorly understood, Butterfly Ray species in the western North Atlantic. Through numerous collaborations with a variety of fishery-independent and dependent operations, laboratories, and museum collections, the studies detailed in this dissertation: (1) address important knowledge gaps in our understanding of the Gymnuridae; (2) reveal discoveries that redefine the global biodiversity of the taxon and the regional biodiversity of fauna in western Atlantic marine ecosystems; (3) identify areas of focus for future research on Butterfly Rays, and (4) provide motivation for increasing efforts to monitor and assess batoid populations in U.S. waters.

LITERATURE CITED

Alkusairy, H., Ali, M., Saad, A., Reynaud, C. & Capapé, C. (2014). Maturity, reproductive cycle, and fecundity of spiny butterfly ray, *Gymnura altavela* (Elasmobranchii: Rajiformes: Gymnuridae), from the coast of Syria (eastern Mediterranean). *Acta Ichthyologica et Piscatoria* **44**(3), 229–240.

Bigelow, H. B. & Schroeder, W.C. (1953). Fishes of the Western North Atlantic. Sawfishes, Guitarfishes, Skates, Rays, and Chimaeroids, No. 1, Part 2. Yale University Press. Memoirs Sears Foundation for Marine Research, Yale Univ., New Haven, CT.

Bini, G. (1967). Atlante dei pesci delle coste italiane. I. Leptocardi, Ciclostomi, Selaci. Mondo Sommerso, Milano, 206 pp.

Bizzarro, J. J. (2005). Fishery biology and feeding ecology of rays in Bahia Almejas, Mexico. Master's Thesis, Moss Landing Marine Laboratories, The California State University, San Francisco.

Bornatowski, H., Wosnick, N., Do Carmo, W. P. D., Corrêa, M. F. M. & Abilhoa, V. (2014). Feeding comparisons of four batoids (Elasmobranchii) in coastal waters of southern Brazil. *Journal of the Marine Biological Association of the United Kingdom* **94**, 1491–1499. doi:10.1017/S0025315414000472

Bräutigam, A., Callow, M., Campbell, I. R., Camhi, M.D. and others (2015). Global priorities for conserving sharks and rays: a 2015–2025 strategy. http://fscdn.wcs.org/2016/02/10/1cxcak0agd_GSRI_GlobalPrioritiesForConservingSharksAndRays_web_singles.pdf

Brander, K. (1981). Disappearance of common skate *Raja batis* from the Irish Sea. *Nature* **290**, 48–49.

Capapé C., Zaouli J., Tomansini J. A. & Bouchereau, J. L. (1992). Reproductive biology of the spiny butterfly ray, *Gymnura altavela* (Linneaus, 1758) (Pisces: Gymnuridae) from off the Tunisian coasts. Sci Mar **56**, 347–355.

Compagno, L. J. V. & Last, P. R. (1999). Gymnuridae. In *FAO species identification guide for fishery purposes. The living marine resources of the Western Central Pacific. Volume 3. Batoid fishes, chimaeras and bony fishes part 1 (Elopidae to Linophyrnidae)* (Carpenter, K. E. & Niem, V. H., eds), pp. 1506–1511. Rome: Food and Agriculture Organization.

Daiber, F. C. & Booth, R.A. (1960). Notes on the biology of the butterfly rays, *Gymnura altavela* and *Gymnura micrura*. *Copeia* 1960, 137–139.

Dulvy, N. K. and Reynolds, J. D. (2002). Predicting Extinction Vulnerability in Skates. *Conservation Biology* **16**, 440–450. doi:10.1046/j.1523-1739.2002.00416.x

Dulvy, N. K., Fowler, S. L., Musick, J. A., Cavanagh, R.,D., Kyne, P. M., Harrison, L. R., Carlson, J. K., Davidson, L. N., Fordham, S. V., Francis, M. P., Pollock, C. M., Simpfendorfer, C. A., Burgess, G. H., Carpenter, K. E., Compagno, L. J., Ebert, D. A., Gibson, C., Heupel, M. R., Livingstone, S. R., Sanciangco, J. C., Stevens, J. D., Valenti, S. & White, W. T. (2014). Extinction risk and conservation of the world's sharks and rays. *Elife*, Cambridge 3:e00590. doi:10.7554/eLife.00590

Ebert, D. A. & Stehmann, M. F. W. (2013). Sharks, batoids, and chimeras of the North Atlantic. FAO Species Catalogue for Fishery Purposes, No. 7. Rome, Food and Agriculture Organization.

Grubbs, R. D. & Ha, D. S. (2006). *Gymnura micrura*. The IUCN Red List of Threatened Species. Version 2014.2. www.iucnredlist.org. Downloaded on 23 September 2014.

Heithaus, M. R., Frid, A., Wirsing, A. J. & Worm, B. (2008). Predicting ecological consequences of marine top predator declines. *Trends in Ecology & Evolution* **23**, 202−210.

Heithaus, M. R., Frid, A., Vaudo, J. J., Worm, B. & Wirsing, A. J. (2010). Unraveling the ecological importance of elasmobranchs. In *Sharks and Their Relatives II: Biodiversity, Adaptive Physiology, and Conservation* (Carrier, J. C., Musick, J. A., Heithaus, M. R., eds), pp. 607–633. Boca Raton, FL: CRC Press.

Jacobsen, I. P. & Bennett, M. B. (2009). A taxonomic review of the australian butterfly ray *Gymnura australis*, (Ramsay & Ogilby, 1886) and other members of the family Gymnuridae (Order Rajiformes) from Indo-West Pacific. *Zootaxa* **2228**, 1–28.

Jacobsen, I. P. & Bennett, M. B. (2013). A comparative analysis of feeding and rophic level ecology in stingrays (Rajiformes: Myliobatoidei) and electric rays (Rajiformes: Torpedinoidei). *PLoS ONE* **8**(8), e71348. Doi:10.137/journal.pone.0071348

Kyne, P. M., Carlson, J. K., Ebert, D. A., Fordham, S. V., Bizzarro, J. J., Graham, R. T., Kulka, D. W., Tewes, E. E., Harrison, L. R. & Dulvy, N. K. (2012). The conservation status of North American, Central American, and Caribbean chondrichthyans. Vancouver, Canada: IUCN Species Survival Commission Shark Specialist Group, 156p.

Last, P. R. & Stevens, J. D. (2009). Sharks and Rays of Australia. Melbourne: CSIRO Publishing.

Last, P. R., Naylor, G. J. P. & Manjaji-Matsumoto, B. M. (2016). A revised classification of the family Dayatidae (Chondrichthyes: Myliobatiformes) based on new morphological and molecular insights. *Zootaxa* **4139**(3), 345-368. http://doi.org/10.11646/zootaxa.4139.3.2.

Mandelman, J. W., Cicia, A. M., Ingram, G. W., Driggers, W. B., Coutre, K. M. & Sulikowski, J. A. (2012). Short-term post-release mortality of skates (family Rajidae) discarded in a western North Atlantic commercial otter trawl fishery. *Fisheries Research* **139**, 76–84.

McEachran, J. D. & Capapé, C. (1984). Gymnuridae. In *Fishes of the North-Eastern Atlantic and the Mediterranean, Vol 1*(Whitehead, P. J. P., Bauchot, M.–L., Hureay, J. C., Nielsen, J., Tortonese, E., eds), pp. 203–204. Paris: Unesco.

McEachran, J. D., & de Carvalho, M. R. (2002). Batoid Fishes. In *The living marine resources of the western Central Atlantic. Vol 1 Introduction, molluscs, crustaceans, hagfishes, sharks, batoid fishes and chimaeras. FAO Species Identification Guide for Fisheries Purposes and American Society of Ichthyologists and Herpetologists Special Publication No. 5.* (Carpenter, K. E., ed), pp. 508–589. Rome: Food and Agriculture Organization.

McEachran, J. D. & Séret, B. (1990). Gymnuridae. In *Check-list of the fishes of the eastern tropical Atlantic (CLOFETA), Vol 1. JNICT* (Quero, J. C., Hureau, J. C., Karrer, C., Post, A., Saldanha, L., eds), pp. 64–66. Lisbon: SEI and Paris: UNESCO.

Muktha, M., Akhilesh, K. V., Sandhya, S., Jasmin, F., Jishnudev, M. A. & Kizhakudan, S. J. (2016). Re-description of the longtail butterfly ray, *Gymnura poecilura* (Shaw, 1804) (Gymnuridae: Myliobatiformes) from Bay of Bengal with a neotype designation. *Marine Biodiversity*. doi 10.1007/s12526-016-0552-8

Murdy, E. O. & Musick, J. A. (2013). Fishes of Chesapeake Bay. Baltimore, Maryland: Johns Hopkins University Press.

Raje, S. G. (2003). Some aspects of biology of four species of rays off Mumbai water. *Indian Journal of Fisheries* **50**(1), 89–96.

Robins, C. R. & Ray, G. C. (1986). A field guide to Atlantic coast fishes of North America. Boston, MA: Houghton Mifflin Company, 354 pp.

Rosenberger, L. J. (2001). Pectoral fin locomotion in batoid fishes: undulation versus oscillation. *Journal of Experimental Biology* **204**(Pt 2), 379-394.

Schwartz, F. J. (1984). Sharks, sawfish, skates, and rays of the Carolina. Special Publication, Institute of Marine Sciences. Morehead City, North Carolina: University of North Carolina.

Simpfendorfer, C. A., Heupel, M. R., White, W. T. & Dulvy, N. K. (2011). The importance of research and public opinion to conservation management of sharks and rays: a synthesis. *Marine and Freshwater Research* **62**, 518–527.

Smith, W. D., Bizzarro, J. J., Richards, V. P., Nielsen, J., Marquez-Farias, F. & Shivji, M. S. (2009). Morphometric convergence and molecular divergence: the taxonomic status and evolutionary history of *Gymnura crebripunctata* and *Gymnura marmorata* in the eastern Pacific Ocean. *Journal of Fish Biology* **75**, 761-783.

Stevens, J. D., Bonfil, R., Dulvy, N. K. & Walker, P. A. (2000). The effects of fishing on sharks, rays, and chimaeras (chondrichthyans), and the implications for marine ecosystems. *ICES Journal of Marine Science* **57**, 476–494.

Sulikowski, J. A., Kneebone, J. & Elzey, S. (2005). Age and growth estimates of the thorny skate (*Amblyraja radiata*) in the western Gulf of Maine. *Fisheries Bulletin* **103**, 161–168.

Vooren, C. M., Piercy, A. N., Snelson, Jr. F. F., Grubbs, R. D., Notarbartolo di Sciara, G. & Serena, S. (2007). *Gymnura altavela*. The IUCN Red List of Threatened Species 2007: e.T63153A12624290

http://dx.doi.org/10.2305/IUCN.UK.2007.RLTS.T63153A12624290.en. Accessed on 07 February 2017

Walls, R. H. L., Vacchi, M., Notarbartolo di Sciara, G., Serena, F., Dulvy, N. K. (2016). *Gymnura altavela*. The IUCN Red List of Threatened Species 2016: e.T63153A16527909. Accessed 07 February 2017

Yokota, L., Goitein, R., Gianeti, M. D. & Lessa, R. T. P. (2013). Diet and feeding strategy of smooth butterfly ray *Gymnura micrura* in northeastern Brazil. *Journal of Applied Ichthyology* **2013**, 1–5.

Yokota, L., Goitein, R., Gianeti, M. D. & Lessa, R. T. P. (2012). Reproductive biology of the smooth butterfly ray *Gymnura micrura*. *Journal of Fish Biology* **81**(4), 1315–1326.

CHAPTER 2

Age and Growth Assessment of Western North Atlantic Spiny Butterfly Ray *Gymnura altavela* (L. 1758) using Computed Tomography of Vertebral Centra

Abstract

Life history strategies of batoid fishes have evolved within dynamic marine ecosystems. Adaptations in reproductive and developmental biology are paramount to the survival of species, and therefore knowledge of growth rates to maturity is fundamental for identifying constraints on the conservation of populations. The butterfly rays (Myliobatiformes: Gymnuridae) are highly derived batoids with generally low reproductive potentials for which age and growth information remains unknown. In this study we applied high-resolution X-ray computed tomography (HRXCT) to vertebral centra from a myliobatiform for the first time to estimate age, and used a multimodel approach to investigate growth of spiny butterfly ray, *Gymnura altavela*. Estimated ages of the oldest male and female were 11 and 18 yrs at disk widths (WD) 1355 mm and 2150 mm, respectively. Disk width-at-age data were analyzed using three growth models (von Bertalanffy, logistic, Gompertz), and the most parsimonious and empirically supported model was the logistic function with sex treated as a fixed effect on asymptotic disk width (WD_∞) and k parameters. Growth model parameter estimates were (males) WD_∞ = 1285.46 ± 67.27 mm, k = 0.60 ± 0.10, and (females) WD_∞ = 2173.51 ± 129.78 mm, k = 0.27 ± 0.04. Results indicated sexually dimorphic growth patterns, with males growing faster and reaching asymptotic size at earlier ages than females. These age and growth results for *G. altavela* represent the first such study for the genus, and suggest that this species grows at a similar rate as many teleosts and some batoids, which is relatively fast among other chondrichthyans.

Key words Myliobatiformes, Gymnuridae, growth coefficient, HRXCT, logistic growth model

Introduction

Batoids (Chondrichthyes: Batoidea) are a cosmopolitan group of skates and rays for which life history traits remain largely unknown relative to other chondrichthyans and teleosts. Many marine batoids inhabit coastal ecosystems, from shallow estuarine to shelf waters, where their characteristic dorso-ventrally flattened body shapes are adapted to benthic habitats that support diverse prey types such as mollusks, crustaceans, polychaetes, and fishes (McEachran and Dunn 1998; Ebert and Bizzarro 2007; Ebert and Stehmann 2013). Although commercial U.S. fisheries do not target rays, overlapping distributions with fishes of economic importance results in their incidental catch (bycatch) in demersal fisheries (Brander 1981; Stevens et al. 2005; Tamini et al. 2006). In general, low value bycatch is unregulated, poorly monitored, and discarded at sea, impeding evaluation of species-specific landings data and the potential impacts on populations. Common effects of fishing include alterations to the size and age structure of populations that may induce compensatory changes in demographic rates (Walker and Hessen 1996; Walker and Hislop 1998; Frisk et al. 2008; Romine et al. 2013). Nearly 20 % of batoid fishes are threatened with extinction according to The International Union for the Conservation of Nature (IUCN) Red List of Threatened Species (www.iucnredlist.org), and 45 % of species are considered 'Data Deficient' due to inadequate life history information, presenting major challenges for the development of effective management strategies (Dulvy et al., 2014). Consequently, reported increases in global batoid landings have generated management and conservation concerns, highlighting the need for improved monitoring of populations and a better understanding of the life history of these taxa worldwide (Simpfendorfer et al. 2011; Mandelman et al. 2012; Dulvy et al. 2014).

In general, large-bodied batoids tend to grow more slowly, live longer, and produce fewer offspring than smaller species, and females grow larger and at a slower rate than males (Frisk 2010; Fisher et al. 2013). Life history traits that are characteristic of most batoids and other

chondrichthyans lead to increased vulnerability of populations to depletion from overexploitation (Hoenig and Gruber 1990), particularly species with large maximum sizes (Dulvy et al. 2000; Dulvy et al. 2014). As both mesopredators and prey that link upper and lower trophic levels, skates and rays may also play important ecological roles in the structure and dynamics of coastal ecosystems (e.g., Murawski 1991; Heithaus et al. 2010; Bornatowski et al. 2014). Thus, perturbations to coastal batoid populations may also impact the stability and productivity of co-occurring species of ecological and economical value. Improved understanding of size-at-age and growth patterns in batoid fishes is prerequisite to assessing the status of populations and evaluating changes in demographics over time.

The spiny butterfly ray *Gymnura altavela* (Linneaus, 1758) is a large coastal stingray (Myliobatiformes) with an amphi-Atlantic and Mediterranean distribution, inhabiting sandy and muddy substrates of western North Atlantic waters from Massachusetts to Florida (McEachran and Capapé 1984; Robins and Ray 1986; McEachran and Séret 1990; McEachran and de Carvalho 2002). Descriptions of the species in U.S. waters are restricted to spatially- and temporally-limited studies from which few life history parameters have been estimated, despite reported geographical variability in their maximum and maturity sizes, and low (< 10 yr^{-1}) uterine fecundity (Bigelow and Schroeder 1953; Daiber and Booth 1960; Schwartz 1984; Capapé et al. 1992; Henningsen 1996). While it is not targeted by U.S. fisheries, *G. altavela* may be commonly captured and discarded in demersal trawling operations that occur where densities are high. The impact of fishing pressure on post-release survival of this species remains unknown, which greatly limits assessments and inferences regarding population status. Threats to the U.S. population are classified as Least Concern by the International Union for Conservation of Nature (IUCN) (https://www.iucn.org), although *G. altavela* is considered globally Vulnerable due to population declines observed in the Southwest Atlantic and West Africa, and is Critically Endangered off the coast of Brazil and in the Mediterranean (Vooren et al. 2007; Walls et al.

2016). Reliable life history information including age and growth estimates are needed for improved vulnerability assessments of western North Atlantic populations of *G. altavela*.

Batoid vertebral centra offer a measure of somatic growth through the mineralization of nutrients and deposition of growth bands over time (Ridewood 1921). These structures have been used to estimate age and evaluate growth of several taxa within the order Myliobatiformes, including the Dasyatidae (Ismen 2003; Jacobsen and Bennett 2010, 2011; O'Shea et al. 2013), Myliobatidae (Martin and Cailliet 1988), Platyrhinidae (Kume et al. 2008), Rhinopteridae (Smith and Merriner 1987; Neer and Thompson 2005; Fisher et al. 2013), Urolophidae (White et al. 2001, 2002), Urotrygonidae (Mejía-Falla et al. 2014), and Rhinobatidae (White et al. 2014). Despite reported disk widths exceeding 2 m (Bini 1967; Schwartz 1984; Bigelow and Schroeder 1953), there is no information on age and growth for any species of butterfly ray (Gymnuridae), presumably due to difficulties interpreting growth bands in the relatively small and poorly mineralized centra. To provide fundamental age and growth information, there is a need for alternative approaches to the examination of vertebral centra for which conventional methods remain inadequate.

Recent advances in high-resolution X-ray computed tomography (CT) scanning at the microscopic scale (i.e., HRXCT) provide fine-scale three-dimensional models that can be digitally sectioned to reveal the micro-structure of soft and hard tissues, and offer a valid and repeatable method for the analysis of calcified vertebral morphology to estimate age in chondrichthyans (e.g., Geraghty et al. 2012). CT scanning offers a non-destructive alternative to traditional chondrichthyan ageing methods (i.e., serial sectioning of vertebrae). Broadly applied to the study of systematic morphology of vertebrates, CT scanning has become a valuable tool for detailed examination of both fossil (Schultze and Cloutier 1991; Maisey 2001a; Witmer et al. 2008) and extant vertebrates including chondrichthyans (Maisey 2001b; Maisey 2004; Hilton et al. 2015; Moyer et al. 2015). The present study applies HRXCT methods to *Gymnura* vertebral centra to determine the age of 49 western North Atlantic *G. altavela*. Age estimates were then

used to describe growth patterns and provide key growth parameters for this population. Sex-specific weight-at-length relationships were also examined in 119 specimens collected over a four-year period from multiple fishery-independent surveys along the U.S. Atlantic coast. The novel application of HRXCT methods presented here is broadly applicable to other chondrichthyans with poorly mineralized vertebrae for which age information is needed for stock assessments. Results from this study are intended to augment life history knowledge of *G. altavela* for improved assessment of the western North Atlantic population.

Materials and methods

Sample collection and HRXCT analysis

Specimens of *G. altavela* were collected between 2012 and 2016 from fishery-independent trawl and longline surveys of shelf waters in the U.S. western North Atlantic (Fig. 1). Individuals were sexed, measured, and dissected in the field or stored frozen for laboratory processing. Disk width (WD) and disk length (LD) were measured to the nearest mm, and individuals were weighed (W) to the nearest 0.1 g. Complete vertebral columns from the synarcual cartilage to the tail tip were excised from specimens and stored frozen for age analysis. Vertebral columns were later thawed and soaked in hot water for 10 to 15 minutes to enable removal of soft tissues and disarticulation of centra. To identify which centra were ideal for age analysis, a pilot study using a subsample of vertebral columns from seven males (n=3 mature, 4 immature) and eight females (n=3 mature, 5 immature) was conducted. Vertebral columns were completely disarticulated, and each centrum was enumerated and air-dried. Dried whole centrum diameter (C_D) was measured to the nearest 0.1 mm, and the C_D coefficient of variation was calculated for every set of five vertebrae. Variation was smallest among precaudal vertebrae, and centrum numbers 35-40 were the largest across all life stages of both sexes with the exception of

one young-of-year female. Based on these observations, one precaudal centrum between numbers 35 and 40 was selected from the posteriormost abdominal region of 49 specimens and preserved in 70 % ethanol for age analysis (Fig. 2a).

Whole *G. altavela* centra were air dried and imaged with a Zeiss (formerly Xradia) MicroXCT 400 (https://www.zeiss.com/microscopy/int/x-ray.html) at The University of Texas High-Resolution X-ray Computed Tomography Facility. Scans were performed using a Hamamatsu X-ray source set to 70 kV/10 W. Three different protocols were used, yielding resolutions scaled to centrum size and usually accommodating multiple centra within a single scan. The largest centra were scanned using the 0.4X objective, acquiring 1441 views over 360 degrees of rotation at 3s/view with a 0.35mm SiO2 X-ray prefilter, yielding 25.0 micron resolution. Medium-sized centra were also scanned using the 0.4X objective, at 4s/view and with distance between the X-ray source and detector set to yield 14.5 micron resolution. The smallest centra were scanned using the 4X objective and a 0.15 mm SiO2 X-ray prefilter at 1.75s/view, yielding 5.5 micron resolution. Depending on the antero-posterior thickness of the included centra, total number of slices ranged from 491 to 990 per scan (Fig. 2b). Image slices were rendered in three dimensions using the Amira (FEI) software program and visualized using false color to enhance centrum density variations (Fig. 2c–f). Virtual models of whole centra were inspected for structural quality using rotation and transparency controls (Fig. 2c–d). Each virtual centrum was sliced in half along the sagittal plane to assess internal anatomy and calcification patterns (Fig. 2e). A thin virtual section from the sagittal plane was then selected for age determination (Fig. 2f).

Statistical analyses

The relationship between the weight and disk width of 119 individuals (n_{male} = 63, n_{female} = 56) was estimated using the equation:

$$W_i = \alpha_i WD_i^{\beta_i} e^{\varepsilon_i} \quad (1)$$

where for the i^{th} individual (i = 1, 2, …, 119) α_i is a constant, β_i is allometric parameter, and ε_i is the multiplicative error term. Sexual dimorphism was examined by assuming:

$$\begin{pmatrix} \alpha_i \\ \beta_i \end{pmatrix} = \begin{pmatrix} \gamma_{0\alpha} + & \gamma_{1\alpha} Sex_i \\ \gamma_{0\beta} + & \gamma_{1\beta} Sex_i \end{pmatrix} \quad (2)$$

where Sex_i is a binary covariate representing the sex of the i^{th} individual (male coded '0', female coded '1'). Equation (2) implies the parameters $(\gamma_{0\alpha}, \gamma_{0\beta})$ and sums $(\gamma_{0\alpha} + \gamma_{1\alpha}, \gamma_{0\beta} + \gamma_{1\beta})$ define the parameters in equation (1) for males and females, respectively (Kimura 2008). Regression assumptions from preliminary model fits were evaluated using histograms, QQ-plots, and visual inspection of residuals, and results supported a multiplicative error structure (Quinn and Deriso 1999). Accordingly, both sides of equation (1) were log transformed prior to model fitting. Ordinary least squares was used for estimation and four model parameterizations were considered: (1) no sex effect; (2) effect of sex on α_i; (3) effect of sex on β_i; and (4) effect of sex on both α_i and β_i. Model selection was determined by goodness-of-fit mean squared error (MSE) and Akaike's information criterion (Akaike 1973; Burnham and Anderson 2002) corrected for small sample size (AIC_c; Zhu et al. 2009). Model-based predictions of weight-at-disk width were back transformed and bias corrected (Sprugel 1983).

Growth band pairs were defined as one opaque and translucent band pair extending through the intermedialia (I) and into the corpus calcareum (CC; Casey et al. 1985; Brown and Gruber 1988). The first opaque band distal to the focus and associated with a change in the angle

of the CC was defined as the birth band (BB; age = 0 years), and annual deposition of band pairs in centra was assumed. Age was estimated by counting band pairs distal from the BB and extending from one arm of the CC, through the I and across the opposing CC arm (Fig. 3; Cailliet et al. 2006).

Growth band pairs were counted on HRXCT digital sections prepared independently by two readers using Amira. All centrum images were read twice by each reader for training and fluency in growth band identification, followed by two blind independent readings to assign ages to each specimen, and readings were temporally separated by two weeks to reduce bias. Reproducibility of age determinations between and among readers was examined through age-bias regression analysis (Campana et al. 1995), and systematic differences in age assignments were tested using Evans-Hoenig's and Yates continuity corrected McNemar's χ^2 tests of symmetry (Bowker 1948; Hoenig et al. 1995; Evans and Hoenig 1998). Age assignment precision was evaluated by calculating within and between reader: 1) percent agreement:

$$PA = \frac{N_a}{N_r} \cdot 100 \tag{3}$$

where N_a and N_r represent the number of ages agreed upon and read, respectively; 2) coefficient of variation (CV; Chang 1982); and 3) index of average percent error (Beamish and Fournier 1981):

$$IAPE = \frac{1}{N}\sum_{j=1}^{N}\left(\frac{1}{R}\sum_{i=1}^{R}\left(\frac{|a_{ij}-a_j|}{a_j}\right)\right) \times 100\% \tag{4}$$

where a_{ij} is the i^{th} age estimate for the j^{th} individual, a_j is the mean age calculated for the j^{th} individual, N is the total number of individuals aged, and the number of times an individual was aged is represented by R.

One HRXCT image slice from each centrum image stack was converted to a two-dimensional 8-bit TIFF file, and linear measurements of the centrum radius (C_R) from the focus to the marginal edge were obtained using the straight line selection tool in ImageJ (https://imagej.nih.gov/ij). A linear model was fitted to estimate the relationship between C_R and

WD using generalized least squares estimation. Two forms were considered, one with and one without variance modeled as a power function of the mean ($\text{var}(\varepsilon_i) = \sigma^2\left(f(x_i, \beta)\right)^{2\theta}$) to explore and accommodate heterogeneity (Ritz and Streibig 2008). AICc was used for model selection. Due to inadequate seasonal coverage of samples, marginal increment analysis could not be used to assess temporal periodicity in growth band formation.

The relationship between disk width and age of *G. altavela* was investigated using multiple growth models fitted using nonlinear least squares (Thorson and Simpfendorfer 2009). Regression assumptions were evaluated using the graphical methods described above for the weight-at-disk analysis and diagnostics from preliminary model fits supported an additive error structure. Model classes considered included:

von Bertalanffy (VBF1; Beverton and Holt 1957)

$$WD_i = WD_{\infty i}\left(1 - e^{-k_i(t_i - t_{0i})}\right) + \varepsilon_i \tag{5}$$

$$\begin{pmatrix} WD_{\infty i} \\ k_i \end{pmatrix} = \begin{pmatrix} \beta_{0WD} & + & \beta_{1WD_\infty} Sex_i \\ \beta_{0k} & + & \beta_{1k} Sex_i \end{pmatrix} \tag{6}$$

Gompertz (GFF1; Quinn and Deriso, 1999)

$$WD_i = WD_{\infty i} e^{-e^{-k_i(t_i - t_{0i})}} + \varepsilon_i \tag{7}$$

$$\begin{pmatrix} WD_{\infty i} \\ k_i \end{pmatrix} = \begin{pmatrix} \beta_{0WD} & + & \beta_{1WD_\infty} Sex_i \\ \beta_{0k} & + & \beta_{1k} Sex_i \end{pmatrix} \tag{8}$$

and logistic function (LGF1; Ricker 1979)

$$WD_i = \frac{WD_{\infty i}}{1 + e^{b_i - k_i t_i}} + \varepsilon_i \tag{9}$$

$$\begin{pmatrix} WD_{\infty i} \\ k_i \end{pmatrix} = \begin{pmatrix} \beta_{0WD} & + & \beta_{1WD_\infty} Sex_i \\ \beta_{0k} & + & \beta_{1k} Sex_i \end{pmatrix} \tag{10}$$

where for the i^{th} individual (i = 1, 2, …, 49), WD_i is disk width, $WD_{\infty i}$ is theoretical asymptotic disk width, k_i is the instantaneous growth coefficient, b_i is a constant (logistic model), t_i is age, t_{0i} is the theoretical age at zero disk width (von Bertalanffy, Gompertz models), ε_i is an additive error term, and Sex_i is a binary covariate coded as described above for the weight-at-disk width analysis (Kimura 2008). For each growth function, model parameterizations considered included: (1) no sex effect; (2) effect of sex on $WD_{\infty i}$; (3) effect of sex on k_i; and (4) effect of sex on both $WD_{\infty i}$ and k_i. For the von Bertalanffy and Gompertz models, the latter three parameterizations were intended to explore sexual dimorphism under the parsimonious assumption that the theoretical size at zero disk width did not differ among sexes. For the logistic models, parsimony was again invoked by not including the effect of sex on b_i. Parameter estimates for males were directly estimated, while parameters for females required summation of the baseline (i.e., male) estimate and the coefficient of the sex effect. Accordingly, standard errors for female parameter estimates were obtained using the delta method (Seber 1982). Size-at-birth (WD_0) was calculated from the y-intercept of the model chosen for inference with standard errors estimated from the delta method. Model selection was based on biological plausibility and concordance of WD_0, WD_∞, and k parameter estimates, goodness-of-fit (MSE), and AIC_c. All statistical analyses were conducted using R (R Development Core Team, 2016), and results were considered significant at $\alpha < 0.05$ (where applicable).

Results

Gymnura altavela specimens used for the weight-at-disk width analysis ranged in size from 427 – 2150 mm WD_{Female} (0.56 – 80.26 kg W_{Female}) and 506 – 1365 mm WD_{Male} (1.13 – 25.50 kg W_{Male}) (Table 1). Differences in the weight-at-disk width relationship among sexes was not empirically supported and was best described by a function of the form $\log W = \log(2.78 \times 10^{-9}\ WD^{3.17})$ (Table 2; Fig. 4). A significant linear relationship between C_R and WD was

described by the equation $C_R = 7.84WD - 2897.07$ (α = -2897.07 ± 431.13; β = 7.84 ± 0.60; θ = 0.98; ΔAIC_c = 38.82 between parameterizations with and without the variance function, which provided strong support for inclusion of the power of the mean model), and demonstrated that vertebral growth was proportional to body size (Fig. 5). Therefore, use of *G. altavela* centra for age analysis was appropriate.

Reconstructed HRXCT vertebral centra from *G. altavela* revealed interpretable growth band pairs. Pre-birth bands were observed but not consistent in all centra, and the BB was associated with a change in CC angle, followed by a broad translucent band reflecting the first year of growth. Narrow OB and broad TB pairs were readily distinguishable across the I and both arms of the CC in digital sections, however, these tended to become compacted near the growth margin in larger individuals.

Age estimates from the two readers did not differ systematically (Evans-Hoenig's χ^2 = 2.29, P = 0.32; McNemar with Yates continuity correction χ^2 = 1.84, P = 0.17). Percent agreement between readers increased from 60 % (IAPE = 5.88, CV = 8.32) during the first reading to 78 % (IAPE = 3.58, CV = 5.06) during the second reading (Fig. 6a). Within reader agreement was 90 % (IAPE = 1.26, CV = 1.78, Fig. 6b) and 92 % (IAPE = 1.04, CV = 1.48) for reader A and B, respectively. Final ages were assigned to all specimens by consensus, and the oldest ages estimated were 18 years for a 2150 mm WD_{female}, and 11 years for a 1355 mm WD_{male} (Table 1).

The most empirically supported disk width-at-age model was the logistic parameterized with the Sex covariate in both WD_∞ and k (LGF4), which resulted in biologically plausible estimates of WD_∞ for males (1285.46 ± 67.27 mm) and females (2173.51 ± 129.78 mm) relative to observed sizes (1355 and 2150 mm, respectively) (Table 3, 4; Fig. 7). Predicted growth coefficients were k_{male} = 0.60 ± 0.10, k_{female} = 0.27 ± 0.04, and b = 1.19 ± 0.12 (Table 4). Size-at-birth calculated from the logistic parameter estimates was 300.80 ± 33.81 for males, 508.61 ± 49.63 for females, and 404.70 ± 38.72 for the average across sexes. The smallest free-swimming individual observed was 496 mm.

Discussion

Findings from this study demonstrated the first successful application of HRXCT for ageing a large stingray species with relatively small (< 1.0 % WD), weakly calcified vertebral centra, and offer further support for the utility of this alternative method in chondrichthyan ageing studies. Furthermore, we provided the first known estimates of age and growth parameters in the Gymnuridae, advancing critical life history knowledge necessary for assessment of the U.S. western North Atlantic *G. altavela* population. The use of HRXCT-reconstructed models of vertebral centra for age estimation offers considerable advantages over manual sectioning and these have been reviewed by Geraghty et al. (2012). Most notably, vertebral centra are preserved whole and therefore available for comparative studies as new methods are developed with advances in technology, and users have unconstrained control over the manipulation (e.g., section thickness, transparency, contrast, perspective) of digital three-dimensional sections, improving the ability to identify and interpret growth bands. It is important, however, to establish a standardized protocol for the examination of virtual sections to ensure consistency between readers. The greatest disadvantage of this method is cost, which effectively limited the sample size in the present study. Recent efforts to provide open access to digital libraries of HRXCT-scanned specimens (e.g., Digimorph, http://digimorph.org, Accessed: 27 February 2017) may improve future accessibility to this method for use in ageing studies.

Precision of age assignments between and within readers was generally high in the present study, with percent agreement between readers improving from 60 to 78 % between reading trials, and 92 % agreement within one year during both trials. Birth band determination was the greatest contributing factor to reader disagreement, followed by the presence of false bands and the compression of marginal bands in the largest individuals. Marginal increment ratios of centrum growth bands (not presented here) were inconclusive because specimens were

predominantly collected by surveys operating during autumn months, thus precluding the determination of seasonal periodicity in band pair formation. Consequently, ages reported here assume an annual deposition of growth bands, which is common in fish ageing studies (Okamura et al. 2013; Cailliet and Goldman 2004), and has been validated in other batoid species (e.g., Sulikowski et al. 2003; Jacobsen and Bennett 2010). Nevertheless, validation of annual deposition of growth bands is needed to verify age estimates for *G. altavela*.

Sexually-dimorphic changes are observed during ontogeny in *G. altavela.* Neonates are born at approximately the same size and shape, and increase in mass and width at similar rates during early life stages, irrespective of sex. Later, this species demonstrates sex-specific patterns in growth, with males reaching a smaller asymptotic size as females continue to increase in size over a longer lifespan. These results support previously reported sexual dimorphism for *G. altavela* (Bigelow and Schroeder 1953; Capape et al. 1992; Alkusairy et al. 2014), other gymnurids (Raje 2003; White and Dharmadi 2007; Jacobsen et al. 2009), and various other batoid species (Ismen 2003; Smith et al. 2007; Sulikowski et al., 2007). Individual mass increased at a greater rate than width during ontogeny, similar to weight-at-length relationships reported for other stingrays, including other species within the genus *Gymnura* (Cailliet and Goldman 2004; Neer and Thompson 2005; White and Dharmadi 2007; Yokota et al. 2012; Teixeira et al. 2016). The allometric parameter estimated for western North Atlantic *G. altavela* ($\beta = 3.17 \pm 0.04$ S.E.) differed from the range of estimates most recently reported for the Mediterranean population ($\beta =$ 2.795 – 3.028), and may be explained by the smaller size range (300 – 1650 mm WD) of individuals examined from the eastern Atlantic (Başusta et al. 2012; Özbek et al. 2016), differences in maximum size, or variations in rates of growth, among other factors. Understanding intraspecific differences in weight-at-length relationships requires adequate ontogenetic and spatio-temporal coverage of a species (Froese 2006).

To account for changes in rates of batoid growth across juvenile, maturing, and adult life stages, sigmoid functions including logistic and Gompertz growth models have increasingly been

utilized (e.g., Mollet et al. 2002; Dale and Holland 2012; White et al. 2014). However, Smart et al. (2016) found little evidence that sigmoid functions consistently perform better than von Bertalanffy growth models for chondrichthyans in general. Growth of *G. altavela* was best described by logistic and Gompertz models, with the former having the greatest statistical support, while less support was associated with von Bertalanffy models that estimated asymptotic size with low precision. The logistic growth model estimated biologically reasonable values of asymptotic size and size-at-birth observed in this and previous studies of western North Atlantic *G. altavela* (Bigelow and Schroeder 1953; Daiber and Booth 1960). Maximum size observed in the present study (2150 mm WD) is similar to the largest *G. altavela* (2170 mm WD) sampled from the same region prior to 1999 (Wigley et al. 2003), but smaller than 2600 mm WD reported by Schwartz (1984). In the western and eastern Mediterranean, smaller maximum sizes ranging from 1342 - 1650 mm WD are reported for this species (Capapé et al. 1992; Başusta et al. 2012; Alkusairy et al. 2014; Özbek et al. 2016). Consequently, there is uncertainty in the taxonomic status of *G. altavela* from U.S. and Mediterranean waters, and recent molecular evidence suggests that individuals from the coast of Senegal (type locality) may be genetically distinct from U.S. individuals (Naylor et al. 2012; Alkusairy et al. 2014). Thus, broader spatio-temporal sampling and taxonomic evaluation of eastern and western Atlantic populations are needed to better understand variation in the growth patterns of this species (Goldman 2005; Alkusairy et al. 2014).

Growth model results from the present study may be widely applied to other batoid taxa to improve understanding of the life history strategies, ecology, and systematic relationships of this diverse group. Sex-specific growth coefficients estimated by the logistic model for *G. altavela* (k_{male} = 0.60; k_{female} = 0.27) were similar to values reported for both large-bodied (> 2400 mm WD) rays, such as spinetail devilray (*Mobula japonica*; pooled sexes k = 0.28; Cuevas-Zimbrón et al. 2012), and relatively small-bodied (< 1000 mm WD) species including western North Atlantic cownose ray (*Rhinoptera bonasus*; k_{male} = 0.26 – 0.27; k_{female} = 0.19; Fisher et al. 2013), eastern Pacific round stingray (*Urotrygon rogersi*; k_{male} = 0.65; k_{female} = 0.22; Mejía-Falla

et al. 2014) and western Pacific fanray (*Platyrhina sinensis*; k_{male} = 0.56; k_{female} = 0.28; Kume et al. 2008). Female gymnurids have a higher energy demand due to matrotrophy, likely resulting in their slower growth rates compared to males. Furthermore, the large body size of female *G. altavela* may impart an evolutionary advantage (e.g., larger offspring and higher fecundity) compared to most other batoids. Future investigations into stingray life history strategies across broad geographic scales are needed to identify key parameters (e.g., age at maturity and fecundity) for improved assessments of populations.

Maternal provisioning of nutrients in butterfly rays results in extreme increases in organic matter between the egg and term embryo stages (Ranzi 1934), yielding relatively large-bodied neonates. Size-at-birth calculated from the logistic model parameter estimates was 405 mm WD for pooled sexes, 301 mm WD for males, and 509 mm WD for females. The smallest free-swimming *G. altavela* observed during this study was 496 mm WD, while Wigley et al. (2003) reported a 200 mm WD specimen in the western North Atlantic; however, it is possible that this latter individual was an aborted embryo rather than a free-swimming neonate. Future investigations should focus on trends in energy allocation (including quantifying the magnitude of maternal provisioning over the course of gestation) and physiological responses to environmental influences (e.g., effect of seasonal temperature fluctuations on growth) during ontogeny for improved understanding of growth and longevity in this species.

The present study provides the first known estimates of age and sex-specific growth patterns for any species of *Gymnura*, contributing to the sparse life history data available for western North Atlantic rays. Results from the present study suggest that *G. altavela* displays moderately fast rates of growth and average longevities relative to other ray species for which age and growth information is available, suggesting the potential for reduced vulnerability of the population to depletion from overexploitation relative to slower growing and longer lived taxa. However, certain life history traits are known to increase extinction risk (e.g., large body size, shallow-water residency, and low fecundity) (Dulvy et al. 2014), and may have contributed to the

depletion of stocks in the Mediterranean and southern portion of the western Atlantic. These factors emphasize the need for further monitoring of U.S. populations, as well as investigating the effects of non-target fisheries on post-release survival. Equally important initiatives to identify and preserve habitats essential for parturition and survival of early life stages of *G. altavela* should be included in the development of management strategies for the conservation of biodiversity and preservation of healthy ecosystems along the U.S. Atlantic coast. Collectively, such efforts rely on species-specific data, hence a taxonomic re-evaluation of *G. altavela* is recommended to delineate the species' range of distribution and life history parameters, which is essential for predicting the vulnerability of populations.

Compliance with Ethical Standards

Field work and sampling were conducted in compliance with protocols approved by the College of William & Mary Institutional Animal Care and Use Committee (IACUC). All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. This article does not contain any studies with human participants performed by any of the authors.

Conflict of Interest:

The authors declare that they have no conflict of interest.

Acknowledgments

We thank the staff of the Virginia Institute of Marine Science (VIMS) Survey Programs (ChesMMAP, NEAMAP, VASMAP, Juvenile Fish and Blue Crab Survey) and the Northeast Fisheries Science Center Multispecies Bottom Trawl Survey for providing specimens for this study. We are grateful to Captains Jimmy Ruhle, Durand Ward and John Olney Jr. for their contributions to vessel operations, and John Galbraith for ensuring access to exceptionally large specimens. We also recognize Julia White and Kamila Aguiar Gabaldo for their assistance with processing vertebrae and HRXCT images. Thanks to M. Kolmann, J. McDowell, and E. Hilton for reviewing previous versions of this paper. Funding for VIMS Survey Programs was provided by: NOAA Chesapeake Bay Office, the Virginia Environmental Endowment, the U.S. Fish and Wildlife Service, and the Virginia Marine Resources Commission (ChesMMAP); the Atlantic States Marine Fisheries Commission, the Mid Atlantic Fisheries Management Council, the Commercial Fisheries Research Foundation, and the Northeast Fisheries Science Center (NEAMAP); NOAA Fisheries (Silver Spring, MD) (VASMAP); the U.S. Fish and Wildlife Service and the Virginia Marine Resources Commission (Juvenile Fish and Blue Crab Survey). This is contribution number xxxx of the Virginia Institute of Marine Science, College of William & Mary.

Literature cited

Akaike H (1973) Information theory as an extension of the maximum likelihood. In: Petrov BN, Csaki F (eds) Proceedings of the Second International Symposium on Information Theory. Akademiai Kiado, Budapest, pp 267–228

Alkusairy H, Ali M, Saad A, Reynaud C, Capapé C (2014) Maturity, reproductive cycle, and fecundity of spiny butterfly ray, *Gymnura altavela* (Elasmobranchii: Rajiformes: Gymnuridae), from the coast of Syria (eastern Mediterranean). Acta Ichthyol Piscat 44(3):229–240

Başusta A, Başusta N, Sulikowski JA, Driggers WB, Demirhan SA, Cicek E (2012) Length-weight relationships for nine species of batoids from the Iskenderun Bay, Turkey. J Appl Ichthyol 28(5):850–851

Beverton RJH, Holt SJ (1957) On the dynamics of exploited fish populations. United Kingdom Ministry of Agriculture and Fisheries, Fisheries Investigations (Series 2) 19, p 533

Beamish RJ, Fournier DA (1981) A method for comparing the precision of a set of age determinations. Can J Fish Aquat Sci 38:982–983

Bigelow HB, Schroeder WC (1953) Fishes of the Western North Atlantic. Sawfishes, Guitarfishes, Skates, Rays, and Chimaeroids, No. 1, Part 2. Yale University Press. Memoirs Sears Foundation for Marine Research, Yale Univ., New Haven, CT

Bini G (1967) Atlante dei pesci delle coste italiane. I. Leptocardi, Ciclostomi, Selaci. Mondo Sommerso, Milano, pp 206

Bornatowski H, Wosnick N, Do Carmo WPD, Corrêa MFM, Abilhoa V (2014) Feeding comparisons of four batoids (Elasmobranchii) in coastal waters of southern Brazil. J Mar Biol Assoc UK 94:1491–1499. doi:10.1017/S0025315414000472

Bowker AH (1948) A test for symmetry in contingency tables. J Am Stat Assoc 43(244):572–574

Brown CA, Gruber SH (1988) Age assessment of the lemon shark, *Negaprion brevirostris*, using tetracycline validated vertebral centra. Copeia 1988:747–753

Brander K (1981) Disappearance of common skate *Raja batis* from the Irish Sea. Nature 290:48–49

Branstetter S, Musick JA (1994) Age and growth estimates for the sand tiger in the Northwestern Atlantic Ocean. Trans Am Fish Soc 123:242–254

Burnham KP, Anderson DR (2002) Model Selection and Multimodel Inference: A Practical Information Theoretic Approach, 2nd edn. Springer, NY

Cailliet GM, Goldman KJ (2004) Age determination and validation in chondrichthyan fishes. In: Carrier J, Musick JA, Heithaus M (eds) Biology of Sharks and Their Relatives. CRC Press, Boca Raton, pp 399–448

Cailliet GM, Smith WD, Mollet HF, Goldman KJ (2006) Chondrichthyan growth studies: an updated review, stressing terminology, sample size sufficiency, validation, and curve fitting. In: Carlson JK, Goldman KJ (eds) Age and Growth of Chondrichthyan Fishes: New Methods, Techniques and Analysis. Springer, Dordrecht, pp 211–228

Campana SE, Annand MC, McMillan JI (1995) Graphical and statistical methods for determining the consistency of age determinations. Trans Am Fish Soc 124:131–138

Capapé C, Zaouli J, Tomansini JA, Bouchereau JL (1992) Reproductive biology of the spiny butterfly ray, *Gymnura altavela* (Linneaus, 1758) (Pisces: Gymnuridae) from off the Tunisian coasts. Sci Mar 56:347–355

Casey JG, Pratt HL, Stillwell C (1985) Age and growth of the sandbar shark (*Carcharhinus plumbeus*) from the western North Atlantic. Can J Fish Aquat Sci 42(5):963–975

Chang WYB (1982) A statistical method for evaluating the reproducibility of age determination. Can J Fish Aquat Sci 39:1208–1210

Conrath CL, Gelsleichter J, Musick JA (2002) Age and growth of the smooth dogfish (*Mustelus canis*) in the northwest Atlantic. Fish Bull 100:674–682

Cuevas-Zimbrón E, Sosa-Nishizaki O, Pérez-Jiménez JC, O'Sullican JB (2012) An analysis of the feasibility of using caudal vertebrae for ageing the spinetail devilray, *Mobula japonica* (Müller and Henle, 1841). Environ Biol Fish 96(8):907–914

Daiber FC, Booth RA (1960) Notes on the biology of the butterfly rays, *Gymnura altavela* and *Gymnura micrura*. Copeia 1960:137–139

Dale JJ, Holland KN (2012) Age, growth and maturity of the brown stingray (*Dasyatis lata*) around Oahu, Hawai'i. Mar Freshw Res 63:475–484

Dulvy NK, Fowler SL, Musick JA, Cavanagh RD, Kyne PM, Harrison LR, Carlson JK, Davidson LN, Fordham SV, Francis MP, Pollock CM, Simpfendorfer CA, Burgess GH, Carpenter KE, Compagno LJ, Ebert DA, Gibson C, Heupel MR, Livingstone SR, Sanciangco JC, Stevens JD, Valenti S, White WT (2014) Extinction Risk and Conservation of the World's Sharks and Rays. Elife, Cambridge 3:e00590. doi:10.7554/eLife.00590

Dulvy NK, Metcalfe JD, Glanville J, Pawson MG, Reynolds JD (2000) Fishery Stability, Local Extinctions, and Shifts in Community Structure in Skates. Conserv Biol 14(1):283–293

Ebert DA, Bizzarro JJ (2007) Standardized diet compositions and trophic levels of skate (Chondrichthyes: Rajiformes: Rajoidei). Env Biol Fish 80(2-3):221–237

Ebert DA, Stehmann MFW (2013) Sharks, batoids, and chimeras of the North Atlantic. FAO Species Catalogue for Fishery Purposes, No. 7. Food and Agriculture Organization, Rome

Evans GT, Hoenig JM (1998) Testing and viewing symmetry in contingency tables, with application to readers of fish ages. Biometrics 54:620–629

Fisher RA, Call GC, Grubbs RD (2013) Age, growth, and reproductive biology of cownose rays (*Rhinoptera bonasus*) in Chesapeake Bay. Mar Coast Fish Dynam Manag Ecosys Sci 5(1):225–234

Frisk MG (2010) Life history strategies of batoids. In: Carrier JC, Musick JA, Heithaus MR (eds) Sharks and Their Relatives II: Biodiversity, Adaptive Physiology, and Conservation. CRC Press, Boca Raton, pp 283–316

Frisk MG, Miller TJ, Martell SJD, Sosebee K (2008) New hypothesis helps explain elasmobranch 'outburst' on Georges Bank in the 1980s. Ecol Appl 18:234–245

Geraghty PT, Jones AS, Stewart J, MacBeth WG (2012) Micro-computed tomography: an alternative method for shark ageing. J Fish Biol 80:1292–1299

Goldman KJ (2005) Age and growth of elasmobranch fishes. In: Musick JA, Bonfil R (eds) Management Techniques for Elasmobranch Fisheries. FAO Fish Tech Pap 474, Rome, pp 97–132

Heithaus MR, Frid A, Vaudo JJ, Worm B, Wirsing AJ (2010) Unraveling the ecological importance of elasmobranchs. In: Carrier JC, Musick JA, Heithaus MR (eds) Sharks and Their Relatives II: Biodiversity, Adaptive Physiology, and Conservation. CRC Press, Boca Raton, pp 607–633

Henningsen AD (1996) Captive husbandry and bioenergetics of the spiny butterfly ray, *Gymnura altavela* (Linnaeus). Zoo Biol 15(2):135–142

Hilton EJ, Schnell NK, Konstantinidis P (2015) When tradition meets technology: systematic morphology of fishes in the early 21st century. Copeia 103(4):858–873

Hoenig JM, Gruber SH (1990) Life-history patterns in the elasmobranchs: implications for fisheries management. NOAA NMFS Technical Report 90. National Marine Fisheries Service, Washington DC, pp 1–16

Hoenig JM, Morgan MJ, Brown C (1995) Analyzing differences between two age determination methods by tests of symmetry. Can J Fish Aquat Sci 52:364–368

Ismen A (2003) Age, growth, reproduction and food of common stingray (*Dasyatis pastinaca* L., 1758) in Iskenderun Bay, the eastern Mediterranean. Fish Res 60:PII S0165-7836(02)00058-9

IUCN Red List (2016) International Union for Conservation of Nature and Natural Resources – IUCN Red List of Threatened Species. http://www.iucnredlist.org/. Accessed 07 February 2017

Jacobsen IP, Bennett MB (2010) Age and growth of *Neotrygon picta*, *Neotrygon annotata* and *Neotrygon kuhlii* from north-east Australia, with notes on their reproductive biology. J Fish Biol 77:2405–2422

Jacobsen IP, Bennett MB (2011) Life history of the blackspotted whipray *Himantura astra*. J Fish Biol 78(4):1249–1268

Jacobsen IP, Johnson JW, Bennett MB (2009) Diet and reproduction in the Australian butterfly ray *Gymnura australis* from northern and north-eastern Australia. J Fish Biol 75:2475–2489

Kimura DK (2008) Extending the von Bertalanffy growth model using explanatory variables. Can J Fish Aquat Sci 65(9):1879–1891

Kume G, Furumitsu K, Yamaguchi A (2008) Age, growth and age at sexual maturity of fan ray *Platyrhina sinensis* (Batoidea: Platyrhinidae) in Ariake Bay, Japan. Fish Sci 74:736–742

Linnaeus C (1758) Systema Naturae, Ed. X. Systema naturae per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis. Tomus I. Editio decima, reformata. Holmiae 1:1–824

Maisey JG (2001a) CT-scan reveals new cranial features in Devonian chondrichthyan "*Cladodus*" *wildungensis*. J Vertebr Paleontol 21:807–810

Maisey JG (2001b) Remarks on the inner ear of elasmobranchs and its interpretation from skeletal labyrinth morphology. J Morphol 250:236–264

Maisey JG (2004) Morphology of the braincase in the Broadnose Sevengill Shark *Notorynchus* (Elasmoobranchii, Hexanchiformes), based on CT Scanning. Am Mus Novit 3429:1–52

Mandelman JW, Cicia AM, Ingram GW, Driggers WB, Coutre KM, Sulikowski JA (2012) Short-term post-release mortality of skates (family Rajidae) discarded in a western North Atlantic commercial otter trawl fishery. Fish Res 139:76–84

Martin LK, Cailliet GM (1988) Age and growth determination of the bat ray, *Myliobatis californica* Gill, in Central California. Copeia 1988:754–762

McEachran JD, Capapé C (1984) Gymnuridae. In: Whitehead PJP, Bauchot M-L, Hureay JC, Nielsen J, Tortonese E (eds) Fishes of the North-Eastern Atlantic and the Mediterranean. Vol 1 Unesco, Paris, pp 203–204

McEachran JD, de Carvalho MR (2002) Batoid Fishes. In: Carpenter KE (ed) The living marine resources of the western Central Atlantic. Vol 1 Introduction, molluscs, crustaceans, hagfishes, sharks, batoid fishes and chimaeras. FAO Species Identification Guide for Fisheries Purposes and American Society of Ichthyologists and Herpetologists Special Publication No. 5. Rome, FAO, pp 508–589

McEachran JD, Dunn KA (1998) Phylogenetic analysis of skates, a morphologically conservative clade of elasmobranchs (Chondrichthyes: Rajidae). Copeia 1998:271–290McEachran JD, Séret B (1990) Gymnuridae. In: Quero JC, Hureau JC, Karrer C, Post A, Saldanha L (eds) Check-list of the fishes of the eastern tropical Atlantic (CLOFETA), Vol 1. JNICT, Lisbon; SEI, Paris; and UNESCO, Paris, pp 64–66

Mejía-Falla PA, Cortés E, Navia AF, Zapata FA (2014) Age and growth of the round stingray *Urotrygon rogersi*, a particularly fast-growing and short-lived elasmobranch. PLoS ONE 9(4): e96077. doi:10.1371/journal.pone.0096077

Mollet, HF, Ezcurra, JM, O'Sullivan, JB, 2002. Captive biology of the pelagic stingray, *Dasyatis violacea* (Bonaparte, 1832). Mar Freshw Res 53:531–541

Moyer JK, Riccio ML, Bemis WE (2015) Development and microstructure of tooth histotypes in the Blue Shark, *Prionace glauca* (Carcharhiniformes: Carcharhinidae) and the Great

White Shark, *Carcharodon carcharias* (Lamniformes: Lamnidae). J Morphol 276:797–817

Murawski SA (1991) Can we manage our multispecies fisheries? Fisheries 16(5):5–13

Natanson LJ (1993) Effect of temperature on band deposition in the little skate, *Raja erinacea*. Copeia 1993:199–206

Neer JA, Thompson BA (2005) Life history of the cownose ray, *Rhinoptera bonasus*, in the northern Gulf of Mexico, with comments on geographic variability in life history traits. Environ Biol Fish 73:321–331

Okamura H, Punt AE, Semba Y, Ichinokawa M (2013) Marginal increment analysis: a new statistical approach of testing for temporal periodicity in fish age verification

O'Shea OR, Braccini M, McAuley R, Speed CW, Meekan MG (2013) Growth of tropical dasyatid rays estimated using a multi-analytical approach. PLoS ONE 8(10): e77194. doi:10.1371/journal.pone.0077194

Özbek EO, Çardak M, Kebapçioğlu T (2016) Spatio-temporal patterns of abundance, biomass and length-weight relationships of *Gymnura altavela* (Linnaeus, 1758) (Pisces: Gymnuridae) in the Gulf of Antalya, Turkey (Levantine Sea). J Black Sea/Mediterranean Environment 21:169–190

Quinn TJ, Deriso RB (1999) Quantitative fish dynamics. Oxford University Press, New York, p 560

R Development Core Team (2016) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Raje SG (2003) Some aspects of biology of four species of rays off Mumbai water. Indian J Fish 50(1):89–96

Ranzi S (1934) Le basi fisio-morfologiche dello sviluppo embrionale dei Selaci. Parti II e III. Pubblication Stazione Zoologica di Napoli 13:331–437

Ricker WE (1979) Growth rates and models. In: Hoar WS, Randall DJ (eds) Fish Physiology. Vol VIII. Academic Press, New York, pp 677-743

Ridewood WG (1921) On the calcification of the vertebral centra in sharks and rays. Phil Trans R Soc B Biol 210:311–407

Ritz C, Streibig JC (2008) Nonlinear Regression with R. Springer, New York: Springer, p 144

Robins CR, Ray GC (1986) A field guide to Atlantic coast fishes of North America. Houghton Mifflin Company, Boston, p 354

Romine JG, Musick JA, Johnson RA (2013) Compensatory growth of the Sandbar Shark in the western North Atlantic including the Gulf of Mexico. Mar Coast Fish 5:189–199

Schultze HP, Cloutier R (1991) Computed-tomography and magnetic resonance imaging studies of *Latimeria chalumnae*. Environ Biol Fish 32:159–181

Schwartz FJ (1984) Sharks, sawfish, skates, and rays of the Carolinas. Special Publication, Institute of Marine Sciences, University of North Carolina, Morehead City

Seber GAF (1982) The estimation of animal abundance and related parameters. 2nd ed. Caldwell, New Jersey: The Blackburn Press, p 654

Simpfendorfer CA, Heupel MR, White WT, Dulvy NK (2011) The importance of research and public opinion to conservation management of sharks and rays: a synthesis. Mar Freshw Res 62:518–527

Smart JJ, Chin A, Tobin AJ, Simpfendorfer CA (2016) Multimodel approaches in shark and ray growth studies: strengths, weaknesses and the future. Fish Fish 17(4):955–971

Smith JW, Merriner JV (1987) Age and growth, movements and distribution of the cownose ray, *Rhinoptera bonasus*, in Chesapeake Bay. Estuaries 10(2):153–164

Sprugel DG (1983) Correcting for bias in log-transformed allometric equations. Ecology 64:209–210

Stevens JD, Walker TI, Cook SF, Fordham S (2005) Threats faced by chondrichthyan fishes. In: Fowler SL, Cavanagh R, Camhi M, Burgess GH, Caillet GM, Fordham S, Simpfendorfer CA, Musick JA (eds) Sharks, rays and chimaeras: the status of the Chondrichthyan fishes. IUCN Species Survival Commission Shark Specialist Group, Gland and Cambridge, pp 48–57

Sulikowski JA, Elzey S, Kneebone J, Jurek J, Huntting Howell W, Tsang PCW (2007) The reproductive cycle of the smooth skate, *Malacoraja senta*, in the Gulf of Maine. Australian Journal of Marine and Freshwater Research 58:98–103

Sulikowski JA, Morin MD, Suk SH, Howell WH (2003) Age and growth of the winter skate, *Leucoraja ocellata*, in the Gulf of Maine. Fish Bull 101:405−413

Tamini LL, Chiaramont GE, Perez JE, Cappozzo HL (2006) Batoids in a coastal trawl fishery of Argentina. Fish Res 77(3):326–332

Teixeira EC, Silva VEL, Fabré NN, Batista VS (2016) Length–weight relationships for four stingray species from the tropical Atlantic Ocean. J Appl Ichthyol 33:594–596

Thorson JT, Simpfendorfer CA (2009) Gear selectivity and sample size effects on growth curve selection in shark age and growth studies. Fish Res 98:75–84

von Bertalanffy L (1938) A quantitative theory of organic growth (inquiries on growth laws II). Hum Biol 10:181–213

Vooren CM, Piercy AN, Snelson Jr. FF, Grubbs RD, Notarbartolo di Sciara G, Serena S (2007) *Gymnura altavela*. The IUCN Red List of Threatened Species 2007: e.T63153A12624290 http://dx.doi.org/10.2305/IUCN.UK.2007.RLTS.T63153A12624290.en. Accessed on 07 February 2017

Walker PA, Hessen HJL (1996) Long-term changes in ray populations in the North Sea. ICES J Mar Sci 53:1085–1093

Walker PA, Hislop JRG (1998) Sensitive skates or resilient rays? Spatial and temporal shifts in ray species composition in the central and north-western North Sea between 1930 and the present day. ICES J Mar Sci 55:392–402

Walls RHL, Vacchi M, Notarbartolo di Sciara G, Serena F, Dulvy NK (2016) *Gymnura altavela*. The IUCN Red List of Threatened Species 2016: e.T63153A16527909. Accessed 07 February 2017

Wigley SE, McBride HM, McHugh NJ (2003) Length-weight relationships for 74 fish species collected during NEFSC Research Vessel Bottom Trawl Surveys, 1992-99. NOAA Tech Memo NMFS NE 171, p 26

White WT, Dharmadi (2007) Species and size compositions and reproductive biology of rays (Chondrichthyes, Batoidea) caught in target and non-target fisheries in eastern Indonesia. J Fish Biol 70:1809–1837

White WT, Hall NG, Potter IC (2002) Reproductive biology and growth during pre- and postnatal life of *Trygonoptera personata* and *T. mucosa* (Batoidea: Urolophidae). Mar Biol 140:699–712

White WT, Platell ME, Potter IC (2001) Relationship between reproductive biology and age composition and growth in *Urolophus lobatus* (Batoidea: Urolophidae). Mar Biol 138:135–147

White J, Simpfendorfer CA, Tobin AJ, Heupel MR (2014) Age and growth parameters of shark-like batoids. J Fish Biol 84:1340–1353

Witmer LM, Ridgely RC, Dufeau DL, Semones MC (2008) Using CT to peer into the past: 3D visualization of the brain and ear regions of birds, crocodiles, and nonavian dinosaurs. In: Endo H, Frey R (eds.) Anatomical Imaging: Towards a New Morphology. Springer-Verlag, Tokyo, pp 67–88

Yokota L, Goitein R, Gianeti MD, Lessa RTP (2012) Reproductive biology of the smooth butterfly ray *Gymnura micrura*. J Fish Biol 81(4):1315–1326

Yokota L, Lessa RP (2007) Reproductive biology of three ray species: *Gymnura micrura* (Bloch & Schneider, 1801), *Dasyatis guttata* (Bloch & Schneider, 1801) and *Dasyatis mariana* (Gomes, Rosa & Gadig, 2000) caught by artisanal fisheries in Northeastern Brazil. Cah Biol Mar 48:249–247

Zhu L, Li L, Liang Z (2009) Comparison of six statistical approaches in the selection of appropriate fish growth models. Chin J Oceanol Limn 27:457–467

Tables

Table 1 Summary of western North Atlantic *Gymnura altavela* used for age assessment. Age groups not represented in this study were 3 – 6 and 14 – 17 for females, and 9 – 10 for males. Weight (W) summary statistics reported here are only for specimens that were aged. For the full weight-at-disk width (WD) analysis, WD and W ranges were: WD_{female} 427 – 2150 mm; W_{female} 0.56 – 80.26 kg; WD_{male} 506 – 1365 mm; W_{male} 1.13 – 25.50 kg

	Age Group (n)	Mean Disk Width (mm)	Range Disk Width (mm)	Mean Wet Weight (kg)	Range Wet Weight (kg)
Female	0 (2)	575.0	544-606	1.6	1.2-1.9
	1 (1)	680		2.8	
	2 (3)	621.7	600-639	1.8	1.5-2.1
	7 (3)	1387.3	1100-1670	17.7	11.3-24.2
	8 (2)	1602.5	1575-1630	37.3	34.8-39.7
	9 (2)	1892.5	1880-1905	64.0	58.0-70.0
	10 (2)	1862.5	1845-1880	66.5	65.1-68.0
	11 (3)	1592.3	1173-1867	49.8	49.0-50.6
	12 (1)	1780.0		68.0	
	13 (1)	2036.0		76.8	
	18 (1)	2150.0		80.3	
Male	0 (1)	561.0		1.5	
	1 (2)	582.5	527-638	1.7	1.2-2.2
	2 (4)	631.8	565-690	2.1	1.5-2.7
	3 (2)	787.5	770-805	4.3	3.9-4.5
	4 (1)	965.0		14.3	
	5 (5)	1196.8	1080-1330	15.7	11.1-20.8
	6 (3)	1094.0	940-1200	13.5	12.8-14.2
	7 (8)	1225.9	1016-1348	19.3	10.4-25.5
	8 (1)	1110.0		11.8	
	11 (1)	1360.0		20.4	

Table 2 Number of parameters (p), mean squared error (MSE), AIC_c, ΔAIC_c, and parameter estimates ± standard errors for models fitted to western North Atlantic *Gymnura altavela* weight-at-disk width data. Model parameterizations were: (1) no covariate; (2) sex covariate on α; (3) sex covariate on β; and (4) sex covariate on α and β. The weight-at-disk width relationship was best described by Model 1 with parameter estimates: $\alpha = 2.54 \times 10^{-9} \pm 6.24 \times 10^{-10}$, $\beta = 3.18 \pm 0.04$

Model	p	MSE	AICc	ΔAICc
1	3	0.0272	-85.23	0.00
2	4	0.0268	-84.43	0.80
3	4	0.0268	-84.51	0.72
4	5	0.0267	-82.61	2.62

Table 3 Number of model parameters (p), mean squared error (MSE × 10^4), corrected Akaike information criterion (AIC_c), and ΔAIC_c for 12 growth models fitted to western North Atlantic spiny butterfly ray disk width-at-age data. For each growth function, model parameterizations considered included (1) no sex effect, (2) effect of sex on $WD_{\infty i}$, (3) effect of sex on k_i, and (4) effect of sex on both $WD_{\infty i}$ and k_i. Parameters estimated without the sex covariate are reported for pooled sexes. The most empirically supported model was the logistic (LGF4) with sexually dimorphic asymptotic disk width and growth coefficient parameters, and a shape parameter for pooled sexes

Model	p	MSE	AIC_c	ΔAIC_c
VBGF1	4	2.91	651.57	23.91
VBGF2	5	1.95	634.52	6.85
VBGF3	5	2.04	636.67	9.00
VBGF4	6	1.85	634.42	6.75
GGF1	4	2.74	648.67	21.01
GGF2	5	1.85	631.97	4.31
GGF3	5	1.89	633.05	5.38
GGF4	6	1.80	633.14	5.48
LGF1	4	2.66	647.24	19.58
LGF2	5	1.85	631.89	4.23
LGF3	5	1.88	632.79	5.13
LGF4	6	1.61	627.66	0.00

Table 4 Parameter estimates ± standard errors from the most empirically supported growth model (LGF4) fitted to western North Atlantic spiny butterfly ray disk width-at-age data. Asymptotic disk width (WD_∞) and the growth coefficient (k) parameters were modeled with a sex covariate, and the shape parameter (b) was modeled for pooled sexes. Disk width-at-birth (WD_0) was derived from the parameter estimates of the LGF4 model.

		WD_∞	k	b	WD_0
LGF4	Pooled			1.19 ± 0.12	404.70 ± 38.72
	Males	1285.46 ± 67.27	0.60 ± 0.10		300.80 ± 33.81
	Females	2173.51 ± 129.78	0.27 ± 0.04		508.61 ± 49.63

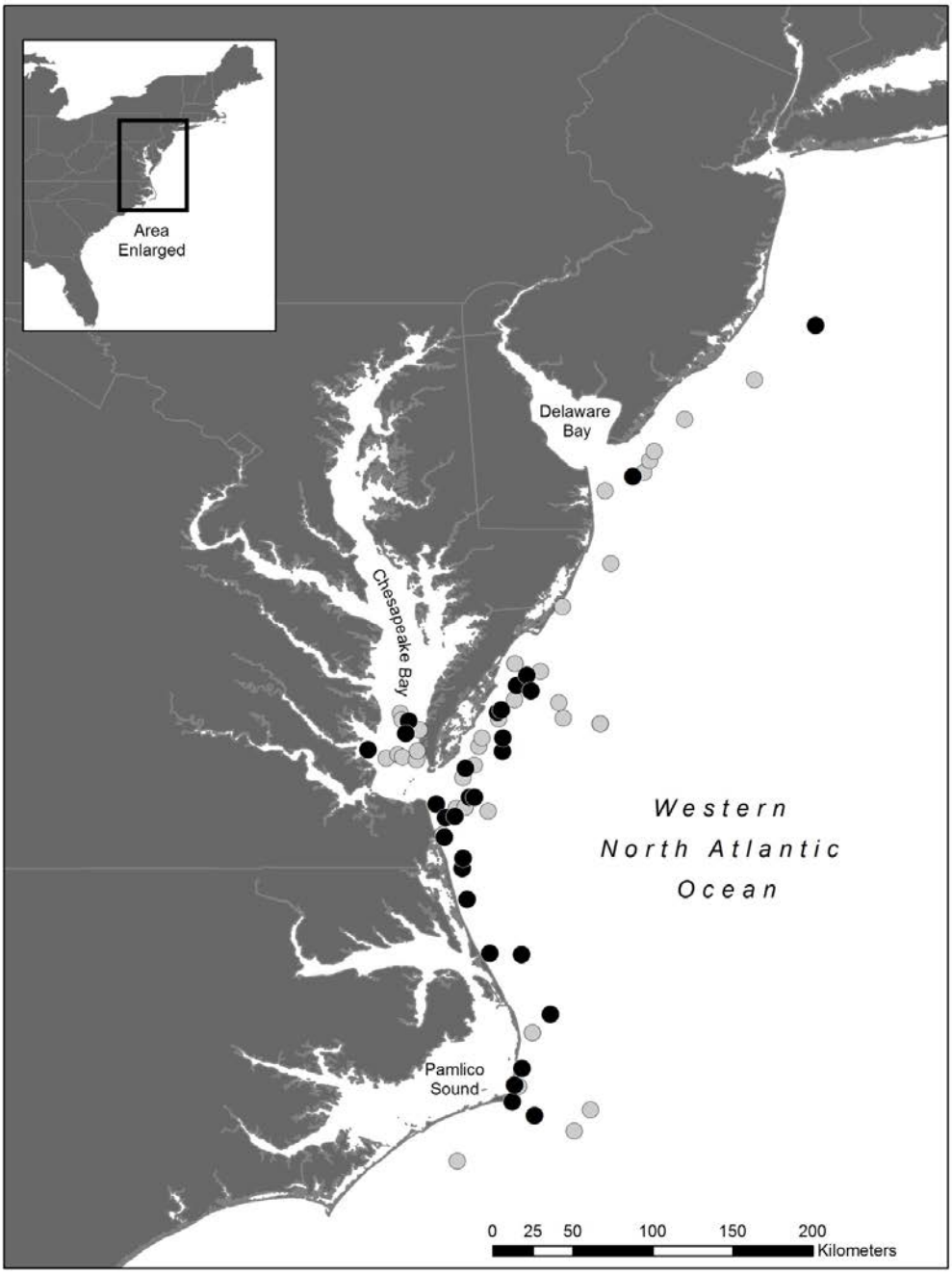


Fig. 1 Map of sampling area and distribution of *Gymnura altavela* specimens collected for this study. Black circles indicate specimens used for age analysis

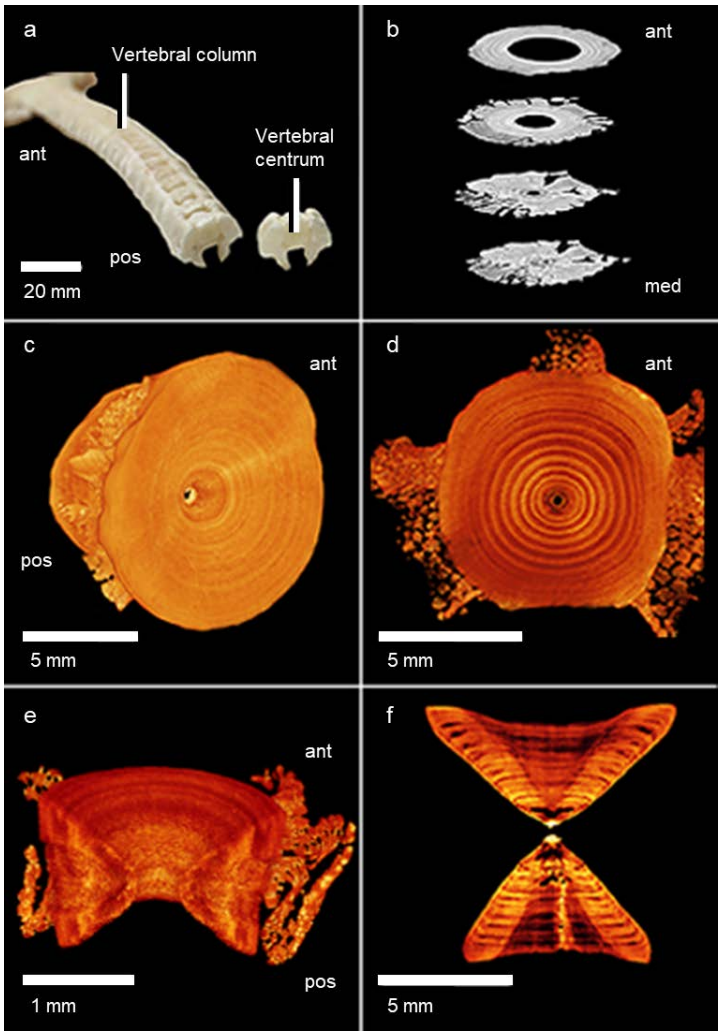


Fig. 2 Methods for vertebral centrum processing for HRXCT scanning, reconstruction, and ageing using Amira software. **a** Vertebral centrum (8.4 mm diameter, female 1905 mm WD) excised from vertebral column, ventral view; **b** examples of slices from reconstructed CT scan of centrum in panel C, anterior (top) to medial (bottom); **c** profile view of false-colored HRXCT-reconstructed whole centrum (8.8 mm diameter, female 1737 mm WD), anterior to right; **d** anterior view of whole centrum (9.6 mm diameter, female 1880 mm WD) adjusted for transparency and contrast to enhance growth band visualization; **e** sagittal plane view of centrum (2.1 mm diameter, female 639 mm WD); **f** sagittal section of centrum in panel C used for age analysis. Ant = anterior, pos = posterior, med = medial

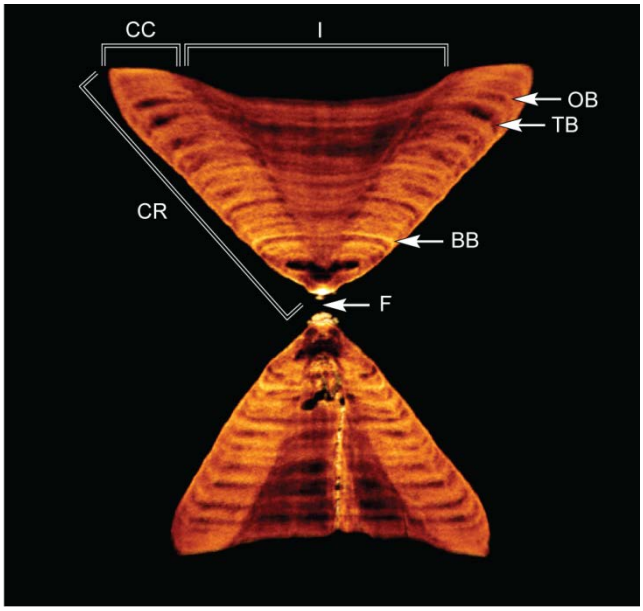


Fig. 3 False-colored HRXCT-reconstructed vertebral centrum section (diameter = 8.8 mm) from female *Gymnura altavela* (1737 mm WD) estimated to be 11 years old. Centrum radius (CR), corpus calcareum (CC), and intermedialia (I) are indicated, and arrows mark the focus (F), birth band (BB), and transparent (TB) and opaque bands (OB)

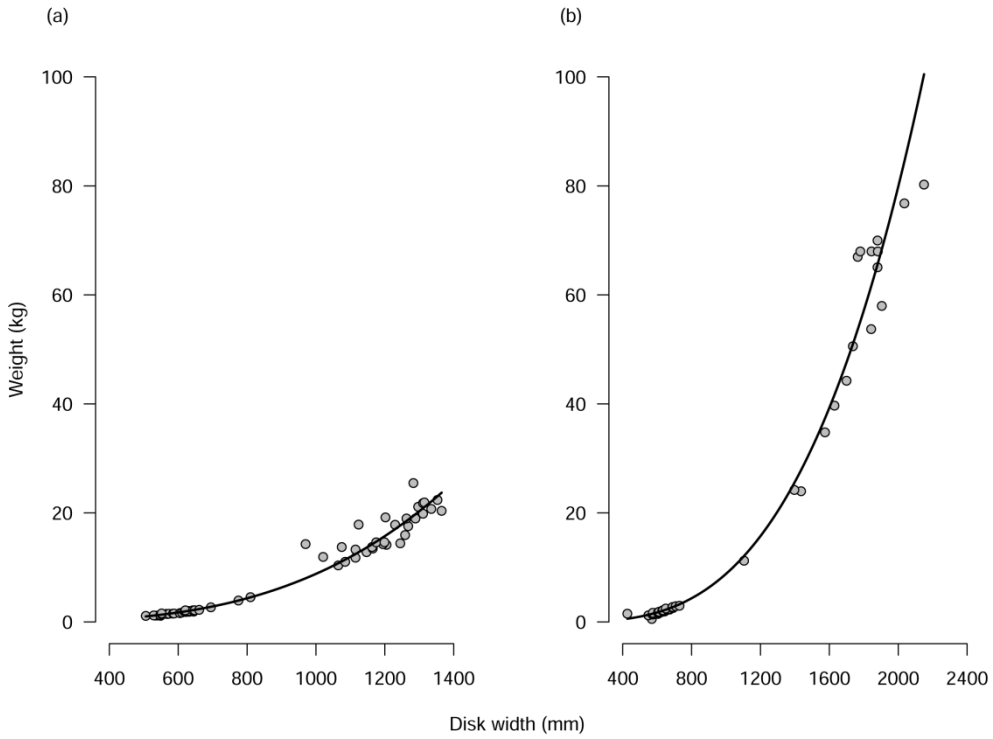


Fig. 4 Weight-at-disk width relationship for (a) male (n = 63) and (b) female (n = 56) western North Atlantic *Gymnura altavela*

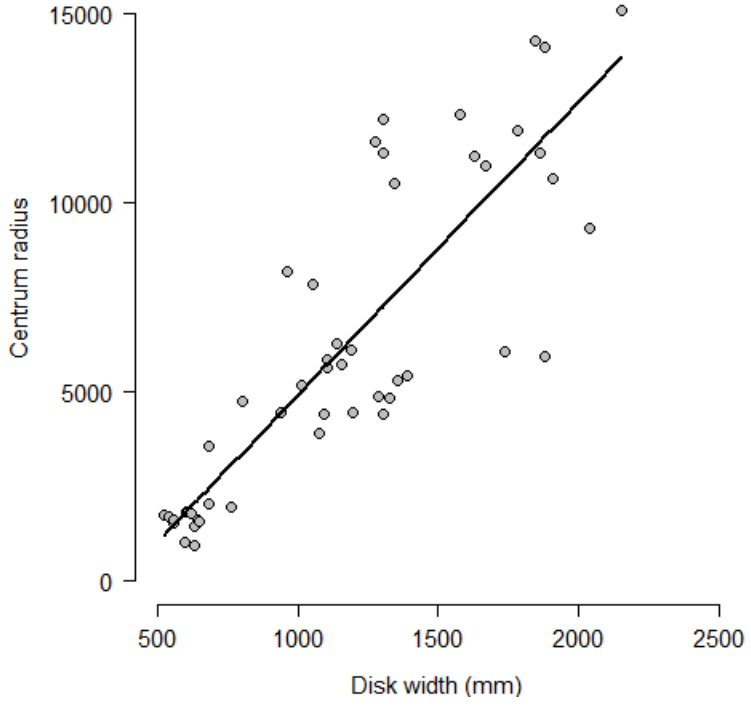


Fig. 5 Centrum radius-at-disk width linear relationship for western North Atlantic *Gymnura altavela* (n = 49)

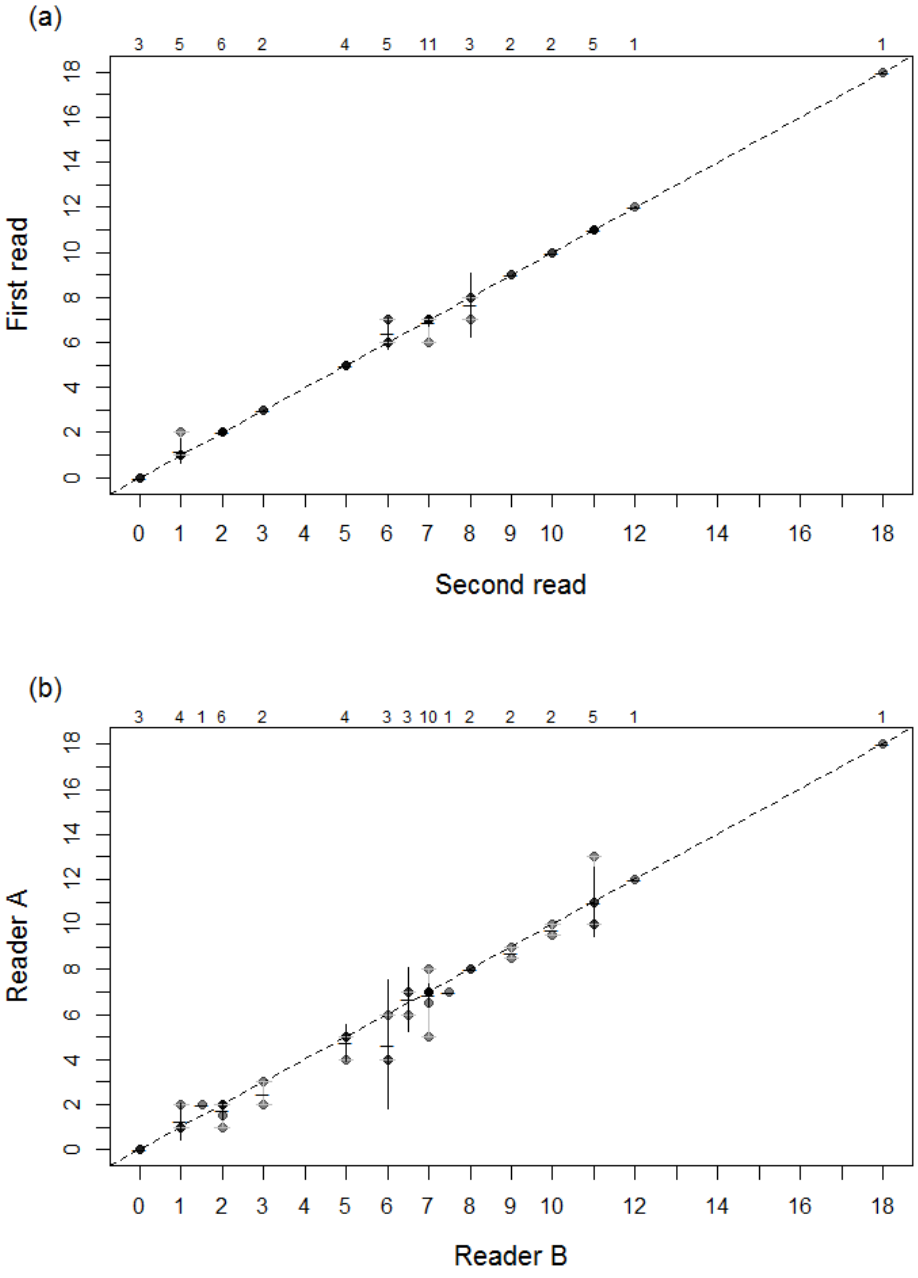


Fig. 6 Age bias plots estimated by (a) reader A and (b) between readers A and B for western North Atlantic *Gymnura altavela* vertebral centra (n = 49). Sample size is indicated on the top axis

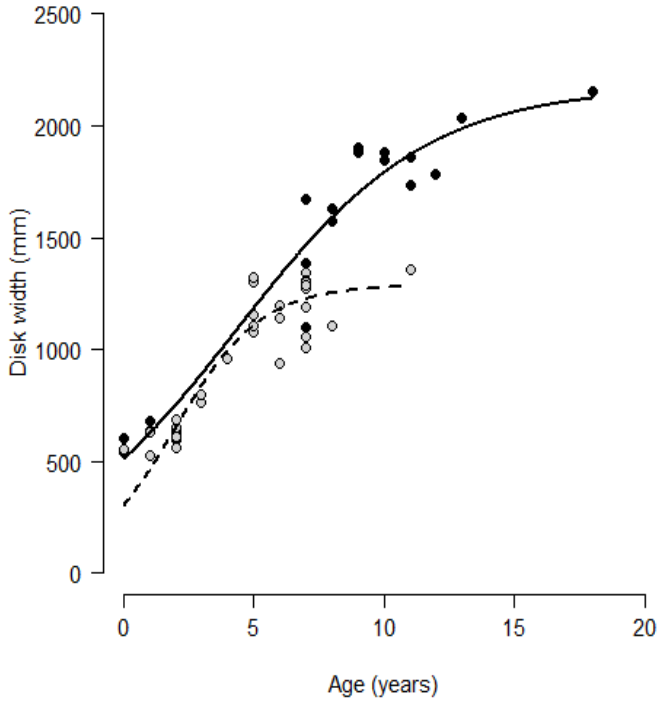


Fig. 7 Logistic growth model fit to disk width-at-age data for male (gray circles, dashed line, n = 28) and female (black circles, solid line, n = 21) western North Atlantic *Gymnura altavela*

CHAPTER 3

Aspects of Reproductive Biology in the Spiny Butterfly Ray (*Gymnura altavela*) and Smooth Butterfly Ray (*Gymnura micrura*) from the Western North Atlantic and Gulf of Mexico

Abstract

The observed maximum disk width (W_D) of *Gymnura altavela* was 2150 mm W_{DF} and 1365 mm W_{DM} for females and males, respectively. Size at reproductive maturity in females (W_{D50F}; 95% C.L.) was estimated to be 1278 mm (1088.0–1467.2), and males reached maturity at smaller sizes (946 mm W_{D50M}; 837.9–1053.8). In the western North Atlantic, *Gymnura micrura* maximum size was 1029 mm W_{DF} and 528 mm W_{DM}, and 544 mm W_{DF} and 364 mm W_{DM} for individuals from the northern Gulf of Mexico. Size at maturity of females and males from the Atlantic population (551 mm W_{D50F}; 526.7–574.5; 390 mm W_{D50M}; 376.7–404.1) was significantly larger than maturity size estimated from the Gulf of Mexico (448 mm W_{D50F}; 398.1–498.3; 298 mm W_{D50M}; 269.8–326.5). Maximum fecundity was seven in *G. altavela*, and ranged from six to 12 in *G. micrura* from the Atlantic and Gulf of Mexico, respectively. Geographic variation in the reproductive biology of *G. micrura* suggests disparate capacities for production in U.S. waters. Thus, a regional-scale approach to the assessment of U.S. butterfly ray populations is recommended, and further investigation into the taxonomic status of the *G. micrura* throughout its distributional range is warranted.

Keywords: Batoid, size-at-maturity, fecundity, matrotrophy histotrophy, trophonemata, gestation

Introduction

The butterfly rays (Myliobatiformes: Gymnuridae) are globally distributed in warm temperate and tropical seas (McEachran, 1982; Compagno *et al*., 1989; Last & Stevens, 2009) where they inhabit shallow coastal regions dominated by sandy and muddy substrates (McEachran & Capapé, 1984; McEachran & Séret, 1990). In the western North Atlantic, two species are recognized in U.S. waters: the spiny butterfly ray, *Gymnura altavela* (Linnaeus 1758), and the smooth butterfly ray, *Gymnura micrura* (Bloch & Schneider 1801). The geographical range of *G. altavela* extends along the U.S. coast from Massachusetts to Florida, with rare occurrences in the Gulf of Mexico (Robins & Ray, 1986; McEachran & Séret, 1990; McEachran & de Carvalho, 2002), while the reported distribution of *G. micrura* is from Maryland to Florida in the Atlantic and extends along the Gulf of Mexico coast from Florida to Texas (Smith, 1997). Both species are also reported from the southwestern and eastern Atlantic (McEachran & Séret, 1990; Ebert & Stehmann, 2013). Significantly larger adult body size, the presence of one or more tail spines, and a tentacle-like lobe on the margin of each spiracle easily distinguishes *G. altavela* from *G. micrura*.

Biological information on *G. altavela* and *G. micrura*, including diet, taxonomy, and reproduction has been reported in several studies (e.g., Daiber & Booth, 1960; Capape *et al*., 1992; Yokota *et al*., 2012; Alkusairy *et al*., 2014). However, investigations into the life history of gymnurid populations in U.S. waters were often spatially and temporally limited, which may bias conclusions due to underrepresentation of some life stages, and lead to inaccurate biological

characterization and taxonomic confusion. Consequently, aspects of the reproductive biology of *G. altavela* and *G. micrura* from the western North Atlantic, including size at maturity, fecundity, and periodicity of reproductive activity, remain fragmented (IUCN, 2013; Last & Stevens, 2009; Henningsen, 1996).

Reproductive anatomy is largely conserved across batoid taxa. Males possess paired testes and external claspers, organs that support spermatogenesis, sperm transport and storage (Hamlett, 1999), and the female anatomy consists of paired ovaries, oviducts, oviducal glands, and uteri, although a variety of specializations relating particularly to uterine accommodation and nutritional support of embryos exists (Hamlett *et al*. 1985, 2005; Hamlett & Kobb, 1999). The Gymnuridae demonstrate lipid histotrophy, in which embryonic development is initially supported by yolk-sac nutrients, and followed by maternal supplementation of protein- and lipid-rich histotroph secreted from uterine trophonemata, a matrotrophic strategy that results in a small number of large offspring (Wourms 1977, 1981; Hamlett & Kobb, 1999). Many elasmobranchs (sharks, skates, and rays) demonstrate slow growth to sexual maturation and relatively low fecundities, traits that increase intrinsic vulnerability of populations to depletion from overexploitation (Holden, 1973; Hoenig & Gruber, 1990; Simpfendorfer & Kyne, 2009). Changes in the age structure, length frequencies, and other demographic rates of batoid populations have been linked to fishing pressure (Brander, 1981; Walker & Hessen, 1996; Walker & Hislop, 1998; Oddone *et al*., 2005), and roughly 20% of species are threatened with extinction (Dulvy *et al*., 2014). A lack of empirical life history data is widespread across chondrichthyans including batoids, of which nearly half (47.5%) of all species are classified as data deficient (Dulvy *et al*., 2014), thus hindering the assessment of populations and development of management strategies.

Gymnurids are incidentally caught in trawls and other benthic fishing gears targeting demersal species in U.S. waters, and high catches are not uncommon in some coastal and

estuarine regions (Shepherd & Myers, 2005; Grubbs & Ha, 2006; K. Parsons, pers. obs.). In the western North Atlantic, *Gymnura* are not considered species of commercial value, and therefore their populations are not managed or directly monitored. Both *G. altavela* and *G. micrura* are considered species of Least Concern in U.S. waters by the International Union for Conservation of Nature and Natural Resources (IUCN) (http://www.iucnredlist.org; Kyne *et al*., 2012). However, widespread population declines of *G. altavela* in other regions have resulted in a global status of Vulnerable, and the species is Critically Endangered in the Mediterranean (Walls *et al*., 2016) and the Southwest Atlantic along the coast of Brazil (Vooren *et al*., 2007). All *G. micrura* populations are considered Data Deficient, and accurate assessments of catches throughout the geographical range of the species are needed (Grubbs & Ha, 2006). Increasing our understanding of batoid life history traits that determine population productivity, such as age and size at maturity, growth rate, fecundity, maximum size, and natural mortality, is a prerequisite for examining the potential for batoid populations to increase or stabilize in response to fishing mortality (Beverton & Holt, 1959; Pauly, 1980), and to rebuild stocks that may be overexploited.

This study investigates the reproductive biology of western North Atlantic *G. altavela* and *G. micrura* through the examination of specimens collected from U.S. fishery-independent trawl surveys with the following objectives: 1) describe sexual dimorphism in body size (i.e., mass-at-disk width relationship, maximum size); 2) estimate spatio-temporal variation in reproductive anatomy (e.g., ovary and testis mass), condition (i.e., gonadosomatic and hepatosomatic indices), periodicity, and fecundity; 3) estimate size at reproductive maturity; and 4) investigate maternal histotrophic supplementation to embryos by measuring changes in organic content throughout development of *G. altavela.* Results from this study address knowledge gaps in the life history of U.S. coastal batoid populations, and highlight the unique and spatio-temporally variable reproductive strategies of *G. micrura*.

Materials and Methods

Specimen collection

Specimens of *Gymnura altavela* and *G. micrura* were collected for life history studies from various fishery-independent trawl surveys conducted between 2012 and 2016 (Fig. 1 and Table I). Specimen disk width (W_D), disk length (L_D), and total length (L_T) was measured to the nearest mm, and individuals were weighed (M) to the nearest 0.001 kg and dissected or stored frozen for laboratory analysis. Dissections were conducted following a standardized protocol: 1) an incision was made at the cloacal opening and along the outer margin of the abdominal cavity to reveal the internal anatomy; 2) the liver was removed and weighed to the nearest g (M_L); 3) reproductive organs were examined to determine maturity status, then removed and weighed to the nearest 0.1 g; and 5) total eviscerated mass was recorded to the nearest g. All samples were blotted dry prior to weighing.

Characteristics of the reproductive system were recorded for all individuals to assign maturity status. Maturity status was determined by macroscopic examination of characters associated with sexual maturity in gymnurids, based on Yokota *et al*. (2012) (Table II and Fig. 2 and Fig. 3). In females, the development of oocytes, oviducal glands, uteri width relative to oviducts, and uterine trophonemata was recorded. Diameters of the five largest ovarian oocytes (D_{O1}–D_{O5}) were measured from the left ovary *in situ*, and the left oviducal gland (D_{OG}) was measured at its widest transverse diameter to the nearest 0.1 mm. Males were assessed by clasper length relative to the posterior margin of the pelvic fin, degree of clasper calcification (i.e., non-, partially-, fully-calcified), enlargement of left testis and lobe development, and the presence of sperm in seminal vesicles. Left and right testes (M_{TL}, M_{TR}) and ovaries ($M_{OL,}$ M_{OR}) were weighed to the nearest 1.0 g.

Uterine contents from gravid females were collected throughout the study period in order to assess reproductive periodicity, fecundity, and gestation in *G. altavela* and *G. micrura*. Uterine eggs and embryos were enumerated and weighed to the nearest 0.001 g, and stored frozen for laboratory analysis of maternal provisioning changes throughout early development in *G. altavela*. Egg diameter (D_E) and embryo disk width (W_{DE}) were measured to the nearest 0.1 mm. Early-stage embryos had external yolk sacs (EYS) larger than the W_D and open gill slits with external gill filaments [Fig. 4(a)]. Mid-stage embryos were characterized by EYS smaller than W_{DE} and the absence of external gill filaments, and late-stage embryos had completely absorbed the EYS but visible remnants of the yolk stalk remained [Fig. 4(b) and (c)]. Full term embryos had only yolk scars present.

Maternal provisioning of nutrients to developing embryos was evaluated in *G. altavela* to better describe and quantify the energetic cost of the matrotrophic reproductive strategy in this taxon as an exemplar for Gymnuridae. Energetic deficit of embryogenesis was determined by comparing ash-free dry mass of fertilized eggs and late-stage embryos to estimate change in organic content throughout gestation. Ash-free dry mass was obtained following a protocol based on Cotton et al. (2015). Samples were dried in aluminum trays at 60°C to constant weight, indicating removal of water content. Dried samples were transferred to a muffle furnace and heated for 4 hours at 150°C, then 12 hours each at 200, 250, 275, and 300°C. Temperatures were then increased to 350°C in 5° increments for 12 hours each. Samples were then incinerated at 550°C for 72 hours. Remaining ash content was weighed to the nearest 0.001 g. Water content (wet mass - dry mass), inorganic content (ash mass), organic content (dry mass - ash mass), and percent increase in organic content from fertilized eggs to late-stage embryos were calculated. All protocols for sampling and euthanizing fish were approved by the College of William & Mary's Institutional Animal Care and Use Committee.

Statistical analyses

The relationship between mass (M) and disk width (W_D) of *G. micrura* was analyzed using the allometric growth equation:

(1)

$$M_i = \alpha_i {W_D}_i^{\beta_i} e^{\varepsilon_i}$$

where for the ith individual α_i is a constant (sometimes referred to as a condition factor), β_i governs curvature, and e^{ε_i} is the multiplicative error term. Sexual dimorphism was examined by including a fixed-effects parameterization (Kimura 2008):

$$\begin{pmatrix} \alpha_i \\ \beta_i \end{pmatrix} = \begin{pmatrix} \gamma_{O\alpha} + & \gamma_{1\alpha} Sex \\ \gamma_{O\beta} + & \gamma_{1\beta} Sex \end{pmatrix}$$

where *Sex* is a binary covariate with intercept and slope parameters $\gamma_{0\alpha}$ and $\gamma_{0\beta}$, respectively. Four parameterizations of the model were fitted using nonlinear least squares: (1) no sex effect, (2) effect of sex on α_i, (3) effect of sex on β_i, and (4) effect of sex on both α_i and β_i.

Maturity status of *G. altavela* and *G. micrura* was categorized into three stages (Stage 1 = immature juvenile; Stage 2 = maturing subadult; Stage 3 = mature adult; Fig. 2). Maturity-at-size was analyzed using a binomial maturity classification (i.e., 0 = immature juveniles and subadults, 1 = mature adults) and a binomial generalized linear model (McCullagh & Nelder, 1989):

(2)

$$logit(p) = \beta_0 + \beta_1 W_D + \beta_2 Sex$$

where p denotes the probability of being mature and the β_i's are estimated parameters. Sizes of 50% maturity (W_{D50}) for males and females were calculated from estimated parameters and the associated 95% confidence intervals were calculated using the delta method (Seber 1982).

Differences between the mass of the left and right gonads were investigated using non-parametric Wilcoxon signed-rank tests with continuity correction to accommodate the non-normal distribution of the difference in mass between left and right gonads. Reproductive and energetic condition of males and females were assessed through evaluation of relationships between body mass, liver mass (M_L), and mass of the left gonad (M_{GL}). Gonadosomatic index (I_G) and hepatosomatic index (I_H) were calculated as:

(3)

$$I_G = \frac{M_{GL}}{M}$$

(4)

$$I_H = \frac{M_L}{M}$$

Where M_{GL}, M_L, and M are as defined above. Confidence intervals for estimated monthly means were derived from 1000 bootstraps. Due to the integration of gonads with the epigonal organ, I_G measurements for males and females included epigonal mass. Since I_G and I_H measurements are proportions, monthly and sex effects were analyzed using beta regression (Ferrari and Cribari-Neto 2004). Seasonal trends in mature female oocyte size (D_{On}, n = oocytes 1 to 5) were investigated using linear mixed effects (LME) models to better understand ovulation cycles. Month and disk width were treated as fixed effects, and to account for the violation of

independence associated with multiple oocyte diameters from the same female, each individual specimen was treated as a random effect (Zuur et al. 2009). Oocyte data were log-transformed when deviations from non-normality and homoscedasticity were detected from diagnostic plots. Fecundity estimates were derived from embryo counts, and the relationship between maternal disk width and uterine fecundity was analyzed by linear regression. Size at birth (W_{DB}) was estimated from the difference between the disk widths of the largest embryos *in utero* and the smallest free swimming young observed during the study.

Evaluation of model assumptions was performed using histograms and QQ-plots of residuals, and homoscedasticity of variables was assessed through visual residual analyses (Quinn and Deriso 1999). The most empirically supported and parsimonious models were selected by negative log likelihood and Akaike's information criterion (AIC; Akaike 1973; Burnham and Anderson 2002) corrected for small sample sizes (AIC_c; Zhu et al. 2009). Differences between relative AIC_c values ($\Delta AIC_c = AIC_c - AIC_{cmin}$) were calculated for each model, and ΔAIC_c values between 0 and 2 indicated substantial empirical support (Burnham and Anderson, 2002). For mixed effects linear model selection, maximum likelihood estimation was utilized for AIC_c comparison, and predicted mean values were estimated using restricted maximum likelihood estimation (REML). Results were considered statistically significant at $\alpha<0.05$ and all statistical analyses were performed using the R software program (R Development Core Team, 2016).

Results

Five hundred and forty-three individuals were examined, comprising 129 specimens of *G. altavela* collected from New Jersey to North Carolina, and 416 specimens of *G. micrura*

obtained from Delaware to eastern Florida in the Atlantic (n = 296), and from Texas, Alabama, and western Florida in the northern Gulf of Mexico (n = 120) (Fig. 1).

Gymnura altavela

Sixty-four female (n_F) and 65 male (n_M) *G. altavela* were sampled during March, June, July, September, October and November between 2012 and 2016. Specimens included individuals that were immature (W_{DF} = 427 – 1397 mm; W_{DM} = 529 – 970 mm) and mature (W_{DF} = 1178 – 2150 mm; W_{DM} = 1021 – 1365 mm) (Table III); two females and two males were of unknown maturity status. The estimated size at which 50% of individuals were mature (W_{D50}; 95% C.I.s) was W_{D50F} = 1277.6 mm; 1088.0 – 1467.2 mm [Fig. 5(a)] and W_{D50M} = 945.8 mm; 837.9–1053.8 mm [Fig. 5(b)] for females and males, respectively. Gonad mass increased asymmetrically, and the lack of development of ovarian follicles and testicular germinal zones in right gonads suggested they were non-functional [Fig. 3(a)]. Left ovary mass ranged from 0.001 – 0.392 kg and was significantly greater than right ovary mass (Wilcoxon test W = 406, z-test Z = 4.69, P = <0.001, r = 0.62), while left testis mass ranged from 0.002 – 0.150 kg and was also significantly greater than right testis mass (W = 946, Z = 5.88, P = <0.001, r = 0.87).

Monthly variation in mean I_G was best described by a model that included the sex covariate ($M1_{IG}$) [Table IV(b)]. Predicted monthly mean I_G for both females [Fig. 6(a)] and males [Fig. 6(b)] decreased significantly from September to October [Table V(b)]. Only one female was sampled in the spring (March) and summer (June) [Fig. 6(a)], and one male was sampled in the winter [Fig. 6(b)]. The most empirically supported model describing monthly variation in I_H did not include the sex covariate ($M2_{IH}$) [Table IV(b)]. A statistically significant decrease in mean I_H occurred from September to October in both females and males [Table V(b) and Fig. 7(a)]. The

highest I_H value was estimated from one female collected in the spring (March: 0.070), while the lowest I_H was estimated from one male collected in fall (November: 0.025) [Fig. 7(a)].

In mature females, D_{OG} ranged from 14.4 – 27.8 mm. The relationship between D_O and month modeled without the individual W_D covariate ($M2_{DO}$) received the most empirical support [Table IV(c)]. A significant relationship between D_O and month was detected, and mean predicted D_O was largest in spring (March: 24.16 mm) when eggs were present, followed by fall when embryos (September: 18.71 mm) and both eggs and embryos (October: 11.25 mm) were observed [Fig. 8(a)]. The smallest mean D_O was predicted in summer (August: 7.7 mm), and coincided with the presence of embryos only [Fig. 8(a)].

Of the 19 mature females, six (1178 – 1905 mm W_D, 49 – 68 kg M) were gravid with four to six uterine eggs, and occurred in North Carolina waters in spring (March, n =1) and in Virginia waters in the summer and fall (June, n = 1; October, n = 4). A maximum of three eggs was observed in a single egg envelope. Average egg fecundity was five and each egg mass ranged between 0.009 – 0.016 kg (n = 21), with D_E between 12.0 and 67.5 mm. The largest D_E were observed from a 1845 mm W_D female in the fall (October: 55.9 – 67.5 mm D_E), and a similar sized female (1843 mm W_D) collected in spring (March: 35.0 – 40.0 mm D_E), while the smallest D_E was observed in a 1880 mm W_D female during the summer (June: 12.0 – 17.0 mm).

Seven females between 1670 and 2036 mm W_D (67 – 77 kg M) gravid with embryos were captured in summer (August) and fall (September and October) in Virginia and North Carolina waters, respectively. Gravid females were collected from water depths between 11.3 and 32.6 m, with bottom temperature and salinity profiles ranging from 19.8 to 21.5°C and 32.9 to 33.8, respectively. Embryo size varied from 172 to 291 mm W_{DE} (0.09 – 0.27 kg M, n = 36) between August and October [Fig. 9(b)]. Embryo sex ratio did not differ significantly from 1:1, and the

number of embryos in the left uterus was often greater than that in the right uterus. Embryo development was most advanced in October, indicated by the near-complete absorption of the EYS and stalk, and skin pigmentation resembling free-swimming individuals [Fig. 4(d)]. Uterine fecundity was between three and seven, and the relationship between maternal W_D and fecundity was not significant (adj. r^2 = 0.073, P = 0.280). The largest mid- to late-stage (i.e. small EYS present) embryo was observed in the fall (October: W_{DE} = 291 mm), and the smallest free-swimming individual was collected in summer (July: W_{DE} = 427 mm), thus the estimated size-at-birth in the western North Atlantic was W_{DE} = 291 – 427 mm.

In freshly fertilized eggs, mean wet mass (9.45 g ± 0.20 S.E.), water content (5.88 g ± 0.14 S.E.), and inorganic content (0.12 g ± 0.00 S.E.) increased in late-stage embryos to 192.22 g ± 6.36 S.E. (+ 1933%), 165.21 g ± 6.05 S.E. (+ 2708%), and 2.66 g ± 0.11 S.E. (+ 2134%), respectively. Wet mass, water content, and inorganic matter slowly increased with the development of mid-stage embryos (W_{DE} < 250 mm), then more rapidly as late-stage embryos (W_{DE} > 250 mm) approached size-at-birth. The change in organic composition between fertilized eggs (3.45 g ± 0.07 S.E.) and late-stage embryos (24.36 g ± 0.64 S.E.) was 606%.

Gymnura micrura – western North Atlantic

A total of 167 female and 129 male *G. micrura* were sampled from the western North Atlantic in all months between April and November. Specimens included 131 immature (W_{DF} = 220 – 595 mm, n_F = 65; W_{DM} = 205 – 450 mm, n_M = 66) and 152 mature (W_{DF} = 506 – 1029 mm, n_F = 95; W_{DM} = 293 – 528 mm, n_M = 57) individuals, and 13 specimens (n_F = 7, n_M = 6) for which maturity status was undetermined (Table III). The relationship between W_D and M varied by sex and was best described by the equation $\log M = \log\left(1.194 \times 10^{-9} {W_D}^{3.34_M, 3.33_F}\right)$, [Table IV(a)

and Fig. 10(a)]. The W_{D50}; 95% C.I. for females was W_{D50F} = 550.62 mm; 526.7–574.5 mm (n_F = 159) [Fig. 5(c)] and W_{D50M} = 390.37 mm; 376.7 – 404.1 mm in males (n_M = 123) [Fig. 5(d)]. Visual inspection of gonads suggested functionality of the left gonad only based on the lack of development of ovarian follicles and testicular germinal zones in the right gonad. Left gonad mass ranged from M_{OL} = 0.0001 – 0.0350 kg and M_{TL} = 0.0001 – 0.0081 kg in females and males, respectively, and was significantly greater than M_{OR} (W = 1324, Z = 5.836, P = 1.453 x 10^{-11}, r = 0.802) and M_{TR} (W = 67, Z = 1.963, P = 0.046, r = 0.401).

The most empirically supported beta regression model fitted to female and male I_G data contained only the model covariate ($M2_{IG}$) [Table IV(b)]. Mean predicted I_G increased from 0.003 in the spring (May) to a peak in summer (July) of 0.005 that was driven by males; a single female collected in July had an I_G 0.21% [Table V(b) and Fig 6(c)]. Mean predicted I_G was relatively stable from late summer through fall (0.003 to 0.004), and then increased again in November to 0.004 (Table V(b) and Fig. 6(c)]. Mean predicted I_H peaked in spring (May: 0.054) and steadily declined throughout the summer to 0.024 in August [Table V(b) and Fig. 7(b)]. In the fall, mean I_H increased from 0.029 to 0.044 between September and October, and then decreased in November (0.042) [Fig. 7(b)].

Oviducal glands in mature females ranged in size from 7.6 – 14.4 mm (n = 55). The relationship between D_O and month was best described without individual W_D as a covariate ($M2_{DO}$) [Table IV(c)]. Mean D_O increased between the spring (May), when only embryos were observed, and early summer (June), during which time both eggs and embryos were present, but decreased in late summer (August) when only eggs were present [Fig. 8(b)]. The largest mean D_O was observed in the early fall (September) in the presence of embryos, and then decreased from October to November, when only eggs were observed [Fig. 8(b)].

Of 95 mature females, uterine eggs were observed in 42 individuals (maternal W_D = 603 – 1029 mm; M = 2.278 – 10.700 kg), and were collected in summer (June and August) from Georgia and Florida, respectively, while the majority of observations occurred in specimens from Delaware, Maryland, Virginia, North Carolina, and Florida during October [Fig. 9(c)]. Fecundity ranged from 2 to 12 with mean masses between 0.001 and 0.005 kg, and D_E ranged from 24.9 to 38.2 mm in October [Fig. 9(d)].

A total of seven gravid females (W_D = 506 – 851 mm, M = 1.295 – 7.271 kg) were collected from water depths between 5.8 and 6.7 m, with bottom temperatures ranging from 24.8 – 31.8°C, and salinities between 28.0 and 28.2. Fecundity varied from 1 to 6 embryos ranging in size from 26.4 – 233.0 mm W_{DE} (M = 0.001 – 0.160 kg, n = 22). Embryos occurred between spring and summer (May and July) in females collected off the east coast of Florida, and in Virginia and Georgia waters during fall (September) and summer (June), respectively. Embryo sex ratio was recorded for a single specimen with one female and four male late-stage embryos. Uterine fecundity did not differ from 1:1 between left and right uteri except for one individual with three embryos in the left uterus and two embryos in the right uterus. Among the embryos examined, the most advanced developmental stage was observed in the fall (Virginia in September), and was indicated by near complete resorption of the EYS and yolk-stalk in five embryos ranging from 227 to 233 mm W_{DE} (M = 0.142 – 0.160 kg) [Fig. 4(e) and Fig. 9(d)]. Maternal W_D did not have a significant effect on fecundity. The smallest free-swimming individuals were observed in summer (August) off the coast of Florida (W_{DE} = 205 mm) and in fall (September) in the Virginia Chesapeake Bay (W_{DE} = 239 mm). Thus, size-at-birth was estimated between 205 – 239 mm W_{DB}, but may vary in the western North Atlantic, with smaller birth sizes possible in southern regions.

Gymnura micrura – Gulf of Mexico

A total of 60 female and 60 male *G. micrura* were sampled from the northern Gulf of Mexico in May, June, July, August, and October. Specimens included 16 immature (W_{DF} = 220 – 595 mm, n_F = 8; W_{DM} = 205 – 450 mm, n_M = 8) and 91 mature (W_{DF} = 260 – 544 mm, n_F = 44; W_{DM} = 242 – 364 mm, n_M = 47) individuals, and 13 individuals (n_F = 8, n_M = 5) for which maturity status was undetermined (Table III). The relationship between W_D and M was best described without the sex covariate by the equation $\log M = \log\left(3.05 \times 10^{-9} {W_D}^{3.18}\right)$ [Table IV(a) and Fig. 10(c) and Fig. 10(d)]. The W_{D50} (95% C.I.) in females was 448.16 mm (398.1 – 498.3) (n_F = 52) [Fig. 5(e)], and 298.18 mm (269.8 – 326.5) in males (n_M = 55) [Fig. 5(f)]. Asymmetrical development of ovaries was observed in specimens from the Gulf of Mexico, with only left ovaries becoming functional and increasing in mass with maturity [Fig. 3(b)]. Differences in testes mass were less pronounced, and while all left testes were functional, some macroscopic structuring of right testes was observed [Fig. 3(d)]. Left ovary mass (M_{OL}) ranged from 0.0001 – 0.0093 kg and was significantly greater than M_{OR} (W = 377, Z = 4.517, P = <0.001, r = 0.869). Mass of left testes ranged from 0.0001 – 0.0029 kg and was not significantly different from M_{TR} (W = 43, Z = 0.981, P = 0.359, r = 0.283).

The most empirically supported beta regression model fitted to female and male I_G data contained a sex covariate ($M1_{IG}$) [Table IV(b)]. Gonadosomatic indices were highest in the spring and fall (May and October) for females (I_{GF} = 0.002) and males (I_{GM} = 0.001), and were lowest in summer (July) (I_{GF} = 0.001; I_{GM} = 0.001) [Fig. 6(d) and Fig. 6(e)]. The most parsimonious and best fitted model describing monthly trends in mean I_H also included a sex covariate ($M1_{IH}$) [Table IV(b)]. Peak hepatosomatic indices occurred in the spring (May) for both sexes (I_{HF} =

0.030 I_{HM} = 0.025) and decreased significantly in the summer (July) (I_{HF} = 0.025; I_{HM} = 0.021) and fall (October) (I_{HF} = 0.021; I_{HM} = 0.018) [Fig. 7(c) and Fig. 7(d)].

In mature females, D_{OG} ranged between 6.0 and 18.9 mm (n = 42). The relationship between D_O and month modeled without individual W_D ($M2_{DO}$) received the most empirical support [Table IV(c)]. Mean D_O was relatively small in Gulf of Mexico specimens, and there was no significant difference between months. When eggs were observed in spring (May), mean D_O was 1.04 mm [Fig. 8(c)]. The smallest D_O were predicted in summer (July; D_O = 1.01 mm) in the presence of both eggs and embryos, while the largest D_O were predicted in the fall (October; D_O = 1.33 mm) when only embryos were observed [Fig. 8(c)].

Uterine eggs were observed during the summer (July) in 19 northern Gulf of Mexico specimens (W_D = 412 – 814 mm; M = 1.24 – 5.49 kg) of *G. micrura* from the Alabama coast. Only one to two eggs were recorded from specimens due to frequent capture-induced abortion. Eggs sampled were generally in poor condition and measurements (i.e., egg diameter and mass) were not possible.

Thirteen *G. micrura* from the Gulf of Mexico between 544 and 856 mm W_D (1.5 – 5.9 kg M) were gravid with one to 12 embryos that ranged in size from 10.4 – 119.9 mm W_{DE} (M = 0.009 – 0.024 kg). Early- (10.4 – 23.5 mm W_{DE}) and mid-stage (37.2 – 73.5 mm W_{DE}) embryos were observed in July off Alabama, and late-stage (98.1 – 119.9 mm W_{DE}) embryos were sampled from two females off the southern Texas coast in October. The distribution of embryos between left and right uteri varied, but neither uteri consistently contained more embryos than the other. Sex ratio data were limited to two litters in which the number of females was greater than males. Remnants of the EYS and stalk (~1 mm total length) remained in the litter of the largest embryos (W_{DE} = 104.6 – 119.9 mm) sampled in October, and full-term embryos were not observed. A

positive and statistically significant relationship between fecundity and maternal W_D was observed ($P = 0.0001$, adj. $r^2 = 0.74$). The smallest free-swimming specimen ($W_D = 242$ mm) was collected in July, thus size-at-birth was estimated between 120 and 242 mm W_{DB}.

Discussion

Detailed information on the reproductive biology of butterfly rays is fragmented due to the patchy spatio-temporal distribution of species and the associated challenges of adequately sampling across ontogeny. This study provides fundamental life history information specific to coastal U.S. species for a better understanding of the population dynamics of *G. altavela* and *G. micrura*.

Gonad development

Gonad asymmetry was observed among gymnurids during this study. The reduction or loss of right or left reproductive structures (i.e., ovaries and testes) is common in viviparous rays (Wourms, 1977), and varies interspecifically among gymnurids (Jacobsen *et al*., 2009). Functional left ovaries and reduced right ovaries observed in *G. altavela* and *G. micrura* are well documented in the literature (Gudger, 1912; Bigelow & Schroeder, 1953; Daiber & Booth, 1960; Capape *et al*., 1992; Snelson *et al*., 1981), whereas both ovaries are functional in the longsnout butterfly ray (*G. crebripunctata*) (Bizzarro, unpub. data) and the California butterfly ray (*G. marmorata*) (Villavicencio-Garayzar, 1993). Similarly, the left testis appeared functional and the right testis was typically reduced in size and undeveloped, a condition previously reported in *G.*

altavela (Daiber & Booth, 1960) and Australian butterfly ray (*G. australis*); this condition may be unique to Gymnuridae among the Myliobatiformes (Jacobsen *et al*., 2009). However, some notable abnormalities were recorded during the present study. One female *G. micrura* collected in June from the coast of Georgia contained a single large (12.2 mm) oocyte in the right ovary that was similar in size and color to left ovary oocytes [Fig. 3(c)]. Among male *G. micrura*, the occurrence of equally sized left and right testes, in which the right testis demonstrated various stages of germinal zone development, was noted in eight specimens from the Atlantic and three from the Gulf of Mexico [Fig. 3(d)]. Histological examination of similarly developed left and right testes was outside the scope of this study, and is needed to determine whether or not functional right testes occur in western Atlantic *G. micrura*.

Maximum size and size-at-maturity

Western North Atlantic *Gymnura* mass-at-disk width relationships were characterized by females growing larger and reaching sexual maturity at larger sizes than males, thus demonstrating sexual dimorphism commonly observed in *Gymnura* and other batoids (Capape *et al*., 1992; Ismen, 2003; Raje, 2003; Smith *et al*., 2007; White & Dharmadi, 2007; Jacobsen *et al*., 2009). Maximum sizes of *G. altavela* (2150 mm W_{DF}; 1365 mm W_{DM}) sampled during this study were similar to those previously reported for the region (2030 – 2170 mm W_{DF}; Bigelow & Schroeder, 1953; Daiber & Booth, 1960; Wigley *et al*., 2003), and larger than sizes observed in the Mediterranean (893 mm W_{DM}, 1342 mm W_{DF}) (Alkusairy, 2014). Off the coast of West Africa, a maximum size of 4000 mm W_D reported by Bini (1967) has not been substantiated and may be erroneous; intense fishing pressure and the removal of large adults from coastal waters of this region since the 1980s, however, was followed by observed decreases in median sizes

(Vooren *et al*., 2007). Therefore, the western North Atlantic population comprises the largest known specimens of *G. altavela* throughout their range of distribution.

Maximum sizes of *G. micrura* were 1029 mm W_{DF} and 528 mm W_{DM} in the Atlantic, and 856 mm W_{DF} and 459 mm W_{DM} in the Gulf of Mexico. Maximum sizes presented here for northern Gulf of Mexico *G. micrura* are the first estimates available for the region. The largest female sizes reported from the Atlantic for the species are 1760 mm W_{DF} (McEachran & de Carvalho, 2002) and 1200 mm W_{DF} (Wigley *et al*., 2003), exceeding female sizes observed in the present study. In the western South Atlantic off the coast of Brazil, a maximum W_D of 660 mm has been reported (Yokota & Lessa, 2007), suggesting that capacity for growth may differ among populations in the northern, temperate regions and southern, tropical waters of the western Atlantic. The influence of temperature on elasmobranch growth and metabolism may culminate in a positive relationship between increases in latitude and body size, commonly referred to as Bergmann's Rule (Mayr, 1942). Slower growth to larger sizes in high latitude relative to low latitude populations has been demonstrated in some western Atlantic batoid species including the little skate (*Leucoraja erinacea*) (Frisk and Miller 2006) and the cownose ray (*Rhinoptera bonasus*) (Neer and Thompson 2005). Considering these findings in addition to the latitudinal size variation in butterfly rays observed in the present study, Bergmann's Rule may explain some mechanisms underlying the dynamics of U.S. coastal batoid populations. Furthermore, compensatory processes resulting from high fishing pressure and removal of large individuals can influence regional vital rates (Walker & Hislop 1998; Frisk & Miller 2006) and may have contributed to maximum size differences reported for *G. altavela* and *G. micrura* between the present and previous studies, among other factors.

Geographic variation in estimated maturity sizes is consistent with differences in maximum sizes. Throughout the Atlantic and Mediterranean, size-at-maturity estimates

previously reported for *G. altavela* range from 961 to 1080 mm W_{DF} and 771 to 1300 mm W_{DM} (Alkusairy *et al*., 2014; Last *et al*., 2016). Our results refine these estimates for western North Atlantic *G. altavela*, and suggest that specimens grow to larger sizes (1278 mm W_{D50F}; 946 mm W_{D50M}) before becoming reproductively mature. Based on age and growth analyses conducted on the sampled population by Parsons *et al*. (in review), the period of growth to sexual maturity is approximately four to seven years.

Likewise, earlier studies documenting maturity size of *G. micrura* suggest a broad range of estimates, from 340 to 813 mm W_{D50F} in females, and 269 to 420 mm W_{D50M} in males, depending on locality (Bigelow & Schroeder, 1953; Daiber & Booth, 1960; McEachran & de Carvalho, 2002; Yokota & Lessa, 2006, 2007; Yokota *et al*., 2012). Maturity sizes estimated here fall within these ranges, with Atlantic coast individuals reaching maturity at larger sizes (551 mm W_{D50F}; 390 mm W_{D50M}) than those sampled from the Gulf of Mexico (448 mm W_{D50F}; 298 mm W_{D50M}). Previous estimates for *G. micrura* occurring along the U.S. coast were limited by lower sample sizes over smaller spatial and temporal scales, thus maturity sizes presented here more accurately represent current populations.

Reproductive periodicity

A number of studies on the reproductive biology of gymnurids suggest temporal variability in annual reproductive and gestation cycles between temperate and tropical regions, largely due to environmental cues that influence the development of gonads and seasonal fluxes in food supply. In tropical regions, consistent prey availability associated with warm and generally stable water temperatures has been proposed to explain year-round, asynchronous reproductive cycles, while seasonal variation in water temperature and dietary resources

characteristic of temperate regions are thought to limit suitable conditions for the survival of early life stages, such that parturition occurs in well-defined periods of the year (Daiber & Booth, 1960; Capape *et al*., 1992).

In the present study, seasonal patterns in oogenesis and the presence and developmental stages of embryos were used to predict reproductive cycles in females. Trends in hepatosomatic and gonadosomatic indices were also examined, as these measures can provide an indication of the energy reserves and general reproductive condition of both males and females. Due to the lack of data for winter and early spring months, the periodicity of reproductive cycles in western North Atlantic *G. altavela* could not be conclusively determined. In the Mediterranean, Capape *et al*. (1992) proposed an annual reproductive cycle for *G. altavela*, with gestation cycles of four to nine months, and parturition occurring at the end of winter. Alkusairy *et al*., (2014) provided further evidence that annual reproductive cycles in the Mediterranean were most likely. This was based on the presence of gravid females in the spring (May) and late-fall and winter (November to December), although the authors state that a biannual cycle could not be discounted. In the western North Atlantic, a six to nine month gestation cycle is reported for the Chesapeake Bay (Murdy & Musick, 2013). Gravid *G. altavela* in this study were observed from August to October, and earlier studies indicate the presence of pregnant females in February and May (Bigelow & Schroeder, 1953; Daiber & Booth, 1960). Only mid-stage and late-stage embryos were observed from late summer (August) to fall (October), and increased from a maximum size of 173 mm to 291 mm W_{DE}, respectively. If size-at-birth occurs between 300 and 427 mm W_{DB}, then parturition may occur in late fall based on the developmental stage of embryos recorded in October. However, the smallest free-swimming individual was sampled in July, providing for the possibility that females also give birth between late spring and early summer months. This is further supported by temporal trends in female I_G, I_H, D_O and the presence of uterine eggs, which

reflect two seasonal peaks in reproductive condition that occur in early spring and fall. Male I_G and I_H was highest in the fall, but was only assessed for the months of July and September through November, thus seasonal patterns could not be discerned. Future sampling of mature specimens during winter and spring months is necessary to resolve the reproductive periodicity of western North Atlantic *G. altavela*.

Gymnura micrura is reproductively active throughout the year in the South Atlantic off the coast of Brazil, as indicated by the presence of embryos during most months and the occurrence of both early- and mid-stage embryos in August (Yokota & Lessa, 2006; Yokota & Lessa, 2007; Yokota *et al*., 2012). The present study reports two peaks in female reproductive condition occur in specimens along the U.S. Atlantic coast, based on maximum I_G and I_H values documented in spring and fall, and large D_O recorded in early summer and mid-fall. The gonadosomatic index of males peaked in summer and fall, while I_H was clearly greatest in fall. However, both indices were highly variable, and annual patterns were unclear since few mature specimens were sampled during winter and early spring months. Uterine eggs were recorded throughout the summer and fall (June to October) in the southwest Atlantic (Yokota *et al*., 2012) and the western North Atlantic (June, August, October and November) (this study). The presence of early- and mid-stage embryos in southern regions of the western North Atlantic (i.e., Georgia and Florida) during late spring and summer (May – July), in addition to presumed young-of-year sampled from Florida in August suggests that parturition may occur in mid- to late-summer in southern regions of the U.S. east coast. In northern regions, late-stage embryos and the smallest free-swimming specimen were observed in fall (September), suggesting parturition occurs in the fall in higher latitudes. Based on these results, a biannual rather than annual reproductive cycle seems most likely, but an annual cycle cannot be excluded. Furthermore, rest cycles in the reproductive periodicity of Brazil specimens have been proposed by Yokota *et al*. (2012) based

on the co-occurrence of gravid females and mature females demonstrating ovarian activity, but lacking embryos. We report a similar case off the Georgia coast in June, when uterine eggs were found to be present in one female at the same time that four females carried embryos. In order to clarify latitudinal variations in the reproductive cycle of western North Atlantic *G. micrura*, improved sampling of the southern Atlantic U.S. coast during summer months is required.

Reproductive cycles for *G. micrura* from the Gulf of Mexico are more difficult to discern due to temporally and spatially limited sampling, with the majority of specimens collected from the Alabama coast between May and July. Florida specimens consisted primarily of mature males, while only two mature and three juvenile females were sampled from Texas. Consequently, conclusions drawn from results presented here should be made with caution and limited to *G. micrura* occurring in the north central Gulf of Mexico.

Off the coast of Alabama, uterine eggs were observed between late spring and summer (May – July), and both early and mid-stage embryos were observed in July but not in May. These results suggest that the onset of embryonic development could occur in late spring; however, it is possible that females gravid with early stage embryos were present during May in the broader region (i.e. northern Gulf of Mexico), but did not occur in the survey area. Embryonic development appears to occur from at least June through October, but seasonal timing of parturition could not be predicted due to the lack of young-of-year and juvenile specimens. Since specimen collection was temporally limited by the sampling frequency of fishery-independent surveys, inferences of seasonal patterns in I_G and I_H could not resolve periodicity of reproductive activity. The co-occurrence of eggs (n = 19) and embryos (n = 11) observed during the summer in the present study reflects the reproductive biology of *G. micrura* in the tropical South Atlantic (Yokota *et al*., 2012), and may be indicative of a rest period in Gulf of Mexico *G. micrura*. Alternatively, the presence of both eggs and embryos could also result from biannual or

asynchronous reproductive cycles in the population, and requires additional seasonal sampling for clarification.

Fecundity and embryonic development

Uterine fecundity in gymnurids typically ranges between three and nine (Jacobsen *et al*., 2009), although 10 to 16 embryos have been observed in *G. marmorata* (Wallace, 1967; Davila-Ortiz, 2002). Maximum fecundity observed in *G. altavela* was seven, confirming the findings of Daiber & Booth (1960). If this species undergoes an annual reproductive cycle and longevity of females is at least 18 (Parsons *et al*., in review), the lifetime productivity of *G. altavela* may range from 30 to more than 80 offspring. The 606% increase in organic content from fertilized eggs to late-stage embryos of *G. altavela* confirms that this is a matrotrophic species, although this value was low compared to increases of 3564% and 4900% between eggs and full term embryos estimated for *G. micrura* (Ranzi, 1934, Yokota *et al*., 2012). Since full-term embryos were not observed, changes in organic content were only estimated for a partial gestation period, accounting for the relatively low percent increase reported here. Other studies that have examined maternal contribution to embryonic growth report a chemical balance of development for *G. altavela* from 22.5 to 30.6 in the Mediterranean (Capape *et al*., 1992; Alkusairy *et al*., 2014); however, these estimates are not directly comparable to the present study due to standard water content values used for eggs (50%) and embryos (75%), which were originally derived from a catshark (Carcharhiniformes: Scyliorhinidae) by Mellinger & Wrisez (1989). Changes in water content of +2708% between eggs and embryos in the present study support the assertion that applying standard water content values across taxa may lead to inaccurate results (Hamlett *et al*., 2005; Braccini *et al*., 2007).

In North Atlantic *G. micrura*, up to eight embryos have been reported (Grubbs & Ha, 2006), while a maximum fecundity of six was recorded in the present study and has also been observed in the South Atlantic off Brazil (Yokota *et al*., 2012). Fecundity was often greater in the Gulf of Mexico than in the Atlantic, with 9 to 12 embryos observed in four specimens, effectively doubling the maximum litter size for the species, and exceeding all records for the genus except *G. marmorata*, which has two functional ovaries (Villavicencio-Garayzar, 1993). Although age, growth and longevity of *G. micrura* has not been reported and reproductive periodicity remains unclear, results from the present study reveal the potential capacity for higher annual rates of production in the Gulf of Mexico relative to the western North Atlantic.

Management and conservation implications

Addressing knowledge gaps and developing effective fisheries management strategies rely on life history data that accurately represent the species for which they are collected, and recent studies suggesting the potential for a large number of undescribed elasmobranchs (e.g. Naylor *et al*., 2012) raise concerns for the taxonomic status of data deficient species, including *G. micrura*. Variations in reproductive parameters between North Atlantic and Gulf of Mexico *G. micrura* in the present study, and previously reported differences between the southwest Atlantic (i.e. Brazil) and western North Atlantic may suggest the existence of distinct populations or species within the western Atlantic (Yokota & Lessa, 2007; Yokota *et al*., 2012). Increased population monitoring and data collection efforts are required for the assessment of many batoid species in general, and a taxonomic review of *G. micrura* is needed in order to (1) assign vital rates to specific populations within their range of distribution, and (2) improve knowledge on the biodiversity of the ecosystems they inhabit (Collette & Vecchione, 1995).

The relatively large body size and low fecundities of *G. altavela* and *G. micrura* suggest the vulnerability of these coastal species to population depletion from overexploitation. Although there are no directed fisheries for gymnurids in U.S. waters, they may commonly be taken as bycatch in demersal trawl fisheries, particularly during mating and pupping seasons when relatively dense but patchy aggregations of large males and females occur in highly productive nearshore areas. Fishing effort during seasons of high productivity along the U.S. east coast may have direct impacts on gymnurid populations, but remain unknown. In elasmobranchs, stress associated abortion of embryos during capture of gravid females may be common (Conrath & Musick, 2012; Trinnie *et al*., 2015). The propensity for gymnurids to abort pre-term embryos during capture can alter reproductive success, since undeveloped embryos likely have a lower probability of survival than full term offspring. Furthermore, the physiological impacts of capture and release have not been evaluated in Gymnuridae, and may have negative effects on the behavior and success of reproductively active individuals during critical periods of the life cycle (i.e., mating and parturition). Investigations into the physiological effects of bycatch practices, including post-release survival, are needed to evaluate the vulnerability of gymnurids in U.S. waters.

Improved monitoring and biological data collection on batoid bycatch must be prioritized to better understand the impacts of fisheries on the health and biodiversity of ecosystems. This study demonstrates the utility of existing fishery-independent research programs as platforms for addressing knowledge gaps in data deficient elasmobranch populations (Collette & Vecchione, 1995), while also highlighting limitations of using survey data that are inherently restricted to the spatio-temporal coverage of sampling designs. Accurate descriptions of the biology of species requires adequate data across both sexes throughout ontogeny in taxa that are sexually dimorphic, since changes in body shape and life stage may manifest in different vulnerabilities to fishing

gears and the impacts of fishing on survival. Without life history information critical for population assessments, precautionary approaches to the management of batoid species that are indirectly affected by fisheries are warranted, particularly for globally declining populations that are considered vulnerable and endangered in parts of their distributional range.

Acknowledgements

This study was depedent on specimens obtained through the cooperative efforts of various agencies, institutions, and laboratories and numerous unnamed individuals. We thank the following groups for their contribution of specimens and data: Northeast Area Monitoring and Assessment Program (NEAMAP), Virginia Shark Monitoring and Assessment Program (VASMAP), Juvenile Fish and Blue Crab Survey, and the Chesapeake Bay Multispecies Monitoring and Assessment Program (ChesMMAP) (Virginia Institute of Marine Science, Department of Fisheries Science); Northeast Fisheries Science Center Multispecies Bottom Trawl Survey; Southeast Area Monitoring & Assessment Program (SEAMAP) Bottom Trawl Survey and the Sea Turtle Trawl Survey (South Carolina Department of Natural Resources, Marine Resources Research Institute); Dauphin Island Marine Laboratory (University of South Alabama, Dauphin Island, AL); Cooperative Atlantic States Shark Pupping & Nursery (COASTSPAN) Bottom Longline Survey (University of North Florida, Shark Biology Program); Fisheries Independent Monitoring Program Bottom Trawl Survey (Florida Fish and Wildlife Research Institute, Jacksonville, FL); Gulf of Mexico Shark Pupping & Nursery (GULFSPAN) Bottom Longline & Gillnet Survey (Florida State University Coastal and Marine Laboratory, St. Teresa, FL); Coastal Fisheries Division Bottom Trawl Survey (Texas Parks & Wildlife Department, Corpus Christi, TX). We especially thank J. Ruhle, D. Ward, J. Gartland , C. Bonzek, J. Galbraith, P. Chase, M. Stratton, G. Mears, C. Ward, K. Spanik, J. Gregg, D. Gregg, D. Gauthier, M. Chattin, T. Tuckey, W. Lowery, E. Loose, J. Olney Jr., V. Hogge, D. Grubbs, M. Kolmann, A. Kroetz, G. Graeber, T. DiGirolamo, R. Brodie, J. Gelsleichter, M. McCallister, L. Parker, J.

Dingle, J. Schwenter, M. Arendt, F. Hernandez, J. White, K. Aguiar Gabaldo, and B. Fisher for the collection or preparation of specimens and data. K. Parsons is grateful to C. Sweetman and C. Peterson for reviewing earlier versions of this chapter.

Literature Cited

Akaike, H. (1973). Information theory as an extension of the maximum likelihood. In *Proceedings of the Second International Symposium on Information Theory*, (Petrov, B. N., Csaki, F. eds), pp. 267–228. Akademiai Kiado, Budapest.

Alcock, A. (1892). Some observations on the embryonic history of *Pteroplataea micrura*. *Annals and Magazine of Natural History* Series 6, **10**, 1–8, pl. 4.

Alkusairy, H., Ali, M., Saad, A., Reynaud, C. & Capapé, C. (2014) Maturity, reproductive cycle, and fecundity of spiny butterfly ray, *Gymnura altavela* (Elasmobranchii: Rajiformes: Gymnuridae), from the coast of Syria (eastern Mediterranean). Acta Ichthyol Piscat **44,** 229–240.

Amoroso, E. C. (1960). Viviparity in fishes. In *Hormones in Fish*, (Jones, I. C. ed), pp. 153–181. Symposia of the Zoological Society no. 1, London.

Beverton, R. J. H. & Holt, S. J. (1959). A review of the lifespans and mortality rates of fish in nature, and their relation to growth and other physiological characteristics. In *CIBA Foundation Colloquia on Ageing: the lifespan of animals*, Vol. 5 (Wolstenholme, G. E. W. & O'Connor, M., eds), pp. 142–180. London: J & A Churchill Ltd.

Bigelow, H. B. & Schroeder, W. C. (1953). Fishes of the western North Atlantic: sawfishes, guitarfishes, skates and rays. *Memoirs Sears Foundation for Marine Research* **1**. New Haven, CT: Yale University Press.

Bini, G. (1967). *Atlante dei pesci delle coste italiane. I. Leptocardi, Ciclostomi, Selaci*. 206 p. Milan: Mondo Sommerso.

Braccini, J. M., Gillanders, B. M., Walker, T.I. & Tovar-Avila, J. (2007). Comparison of deterministic growth models fitted to length-at-age data of the piked spurdog (*Squalus megalops*) in south-eastern Australia. *Marine and Freshwater Research* **58**, 24–33.

Brander, K. (1981). Disappearance of common skate *Raja batis* from the Irish Sea. Nature **290**, 48–49.

Burnham, K. P. & Anderson, D. R. (2002). Model Selection and Multimodel Inference: A Practical Informationtheoretic Approach, 2nd edn. Springer, NY

Capapé, C. (1981). Nouvelle description de *Gymnura altavela* (Linnaeus, 1758) (Pisces. Rajiformes Gymnuridae). *Bulletin Institute National Scientific Technical Oceanographic Peche Salammbo* **8**, 59–68.

Capapé, C., Zaouli, J., Tomansini, J. A. & Bouchereau, J. L. (1992). Reproductive biology of the spiny butterfly ray, *Gymnura altavela* (Linneaus, 1758) (Pisces: Gymnuridae) from off the Tunisian coasts. *Scientia Marina* **56**, 347–355.

Collette, B. & Vecchione, M. (1995). Interactions between fisheries and systematics. *Fisheries* **20**, 20–25.

Compagno, L. J. V., Ebert, D. A. & Smale, M. J. (1989). *Guide to the Sharks and Rays of Southern Africa*. London: New Holland Publishers.

Cotton, C. F. (2010). Age, growth, and reproductive biology of deep-water chondrichthyans. Doctoral dissertation, College of William & Mary, School of Marine Science, Virginia Institute of Marine Science, Williamsburg, VA

Daiber, F. C. & Booth, R. A. (1960). Notes on the biology of the butterfly rays, *Gymnura altavela* and *Gymnura micrura*. *Copeia*, 137–139.

Dávila-Ortiz, J. (2002). Biologiá reproductiva de la raya mariposa *Gymnura marmorata* (Cooper 1863), en Bahía Almejas, B.C.S., México. Thesis de Licenciatura. 38 p.

Dulvy, N. K., Fowler, S. L., Musick, J. A., Cavanagh, R. D., Kyne, P. M., Harrison, L. R., Carlson, J. K., Davidson, L. N., Fordham, S. V., Francis, M. P., Pollock, C. M., Simpfendorfer, C. A., Burgess, G. H., Carpenter, K. E., Compagno, L. J., Ebert, D. A., Gibson, C., Heupel, M. R., Livingstone S. R., Sanciangco, J. C., Stevens, J. D., Valenti, S. & White, W. T. (2014). Extinction risk and conservation of the world's sharks and rays. *Elife*, Cambridge 3:e00590. doi:10.7554/eLife.00590.

Ebert, D. A. & Stehmann, M. F. W. (2013). Sharks, batoids, and chimeras of the North Atlantic. *FAO Species Catalogue for Fishery Purposes*, No. 7. Rome: Food and Agriculture Organization.

Ferrari, S. L. P. & Cribari-Neto, F. (2004). Beta regression for modelling rates and proportions. *Journal of Applied Statistics* **31**, 799–815.

Frisk, M. G. & Miller, T. J. (2006). Age, growth, and latitudinal patterns of two Rajidae species in the northwestern Atlantic: little skate (*Leucoraja erinacea*) and winter skate (*Leucoraja ocellata*). Canadian Journal of Fisheries and Aquatic Sciences **63**, 1078–1091.

Grubbs, R. D. & Ha, D. S. (2006). *Gymnura micrura*. The IUCN Red List of Threatened Species. Version 2014.2. www.iucnredlist.org. Downloaded on 23 September 2014.

Gudger, E. W. (1912). Natural history notes on some Beaufort.N.C. Fishes, 1910–1911. Proceedings of the Biological Society of Washington **25**, 141–156.

Hamlett, W. C. (1999). Male reproductive system. In *Sharks, skates, and rays: the biology of elasmobranch fishes*, (Hamlett, W. C. ed), pp. 444–470. Baltimore, Maryland: The Johns Hopkins University Press.

Hamlett, W. C. & Koob, T. J. (1999). Female reproductive system. In *Sharks, skates, and rays: the biology of elasmobranch fishes*, (Hamlett, W. C. ed), pp. 398–443. Baltimore, Maryland: The John Hopkins University Press.

Hamlett, W. C., Musick, J. A., Eulitt, A. M., Jarrell, R. L., Kelly, M. A. (1996). Ultrastructure of uterine trophonemata, accommodation for Uterolactation, and gas exchange in the southern stingray, Dasyatis Americana. Canadian Journal of Zoology 74:1417-1430.

Hamlett, W. C., Kormarik, C. G., Storrie, M., Serevy, B. & Walker, T. I. (2005). Chondrichthyan parity, lecithotrophy, and matrotrophy. In *Reproductive biology and phylogeny of chondrichthyes*: *sharks, batoids and chimeras*, (Hamlett, W. C. ed), pp. 395–434. Enfield, New Hampshire: Science Publishers.

Henningsen, A. D. (1996). Captive husbandry and bioenergetics of the spiny butterfly ray, *Gymnura altavela* (Linnaeus). *Zoo Biology* **15**, 135–142.

Holden, M. J. (1973). Are long-term sustainable fisheries for elasmobranchs possible? Rapports et Proces-verbaux des Réunions. Conseil International pour l'Éxploration de la Mer **164**, 360–367.

Hoenig, J. M. & Gruber, S. H. (1990). Life-history patterns in the elasmobranchs: implications for fisheries management. *NOAA NMFS Technical Report 90*, pp. 1-16. Washington D.C.: National Marine Fisheries Service.

Ismen, A. (2003). Age, growth, reproduction and food of common stingray (*Dasyatis pastinaca* L., 1758) in Iskenderun Bay, the eastern Mediterranean. Fisheries Research **60**, PII S0165-7836(02)00058-9.

International Union for Conservation of Nature and Natural Resources – IUCN Red List of Threatened Species. http://www.iucnredlist.org/. Accessed 07 February 2017

Jacobsen IP, Johnson JW, Bennett MB (2009) Diet and reproduction in the Australian butterfly ray Gymnura australis from northern and north-eastern Australia. J Fish Biol 75:2475–2489

Jacobsen, I. P. & Bennett, M. B. (2011). Life history of the blackspotted whipray *Himantura astra*. *Journal of Fish Biology* **78**, 1249–1268.

Kyne, P. M., Carlson, J. K., Ebert, D. A., Fordham, S. V., Bizzarro, J. J., Graham, R. T., Kulka, D. W., Tewes, E. E., Harrison, L. R. & Dulvy, N. K. (2012). The conservation status of North American, Central American, and Caribbean chondrichthyans. Vancouver, Canada: IUCN Species Survival Commission Shark Specialist Group, 156p.

Last, P. R. & Stevens, J. D. (2009). Sharks and Rays of Australia. Melbourne: CSIRO Publishing.

Mayr, E. (1942) Systematics and the origin of species. New York, New York: Columbia University Press.

McEachran, J. D. (1982). Chondrichthyes. In *Synopsis and Classification of Living Organisms*, Vol. 2 (Parker, S. P. ed), pp. 831–858. New York, New York: McGraw-Hill.

McEachran, J. D. & Capape, C. (1984). Gymnuridae. In *Fishesof the north-eastern Atlantic and Mediterranean* (Whitehead P. J. P. *et al*., eds), pp. 203–204. Paris: UNESCO.

McEachran, J. D. & B. Séret (1990). Gymnuridae. In *Check-list of the fishes of the eastern tropical Atlantic* (CLOFETA), Vol 1., (Quero, J. C., Hureau, J. C., Karrer, C., Post, A. & Saldanha, L. eds), pp. 64–66. Lisbon: JNICT; Paris: SEI; Paris: UNESCO.

McEachran, J. D., de Carvalho, M. R. (2002). Batoid Fishes. In *The living marine resources of the western Central Atlantic*, *Vol. 1, Introduction, molluscs, crustaceans, hagfishes, sharks, batoid fishes and chimaeras*, (Carpenter, K. E. ed). FAO Species Identification Guide for Fisheries Purposes and American Society of Ichthyologists and Herpetologists Special Publication No. 5, pp. 508–589. Rome, Italy: Food and Agriculture Organization.

Mellinger J. & Wrisez, F. (1989). Biologie et physiologie comparees dudeveloppment de deux selaciens ovipares lesroussettes *Scyliorhinus canicula* et *Scyliorhinus stellaris*. Evolution de la matiere seche, de LÊeauet des ions (Cl-, Na+, K+) dans le vitellus de S.canicula au cours du developpement. *Bulletin dela Société Zoologique de France* **114**, 51–62.

Murdy E. O. & Musick, J. A. (2013). Fishes of Chesapeake Bay. Baltimore, Maryland: Johns Hopkins University Press.

Naylor, G. J. P., Caira, J. N., Jensen, K., Rosana, K., White, W. & Last, P. (2012). A DNA sequence based approach to the identification of shark and ray species and its implications for global elasmobranch diversity and parasitology. *Bulletin of the American Museum of Natural History* No 367, 263pp.

Neer, J. A. & Thompson, B. A. (2005). Life history of the cownose ray, *Rhinoptera bonasus*, in the northern Gulf of Mexico, with comments on geographic variability in life history traits. *Environmental Biology of Fishes* **73**, 321–331.

Needham, J. (1942). Biochemistry and Morphogenesis. Cambridge, Massachusetts: University Press.

Oddone, M. C., Paesch, L. & Norbis, W. (2005). Size at first sexual maturity of two species of rajoid skates, genera *Atlantoraja* and *Dipturus* (Pisces, Elasmobranchii, Rajidae), from the Southwestern Atlantic Ocean. *Journal of Applied Ichthyology* **21**, 70–72.

Pauly, D. (1980). On the interrelationships between natural mortality, growth parameters and mean environmental temperature in 175 fish stocks. *Journal du Conseil international pour l'Exploration de la Mer* **39**, 175–192.

Raje, S. G. (2003). Some aspects of biology of four species of rays off Mumbai water. *Indian Journal of Fisheries* **50**, 89–96.

Ranzi, (1934). Le basi fisio-morfologische dello sviluppo embrionale dei Selaci, Parti II and III. *Pubblicazioni Della Stazione Zoologica di Napoli* **13**, 331–437.

Robins, C. R. & Ray, G. C. (1986). A field guide to Atlantic coast fishes of North America. Boston, Massachusetts: Houghton Mifflin Company 354 pp.

Schwartz, F. J. (1984). Sharks, sawfish, skates, and rays of the Carolina. Special Publication, Institute of Marine Sciences. Morehead City, North Carolina: University of North Carolina.

Seber, G. A. F. (1982). The estimation of animal abundance and related parameters. 2nd ed. Caldwell, New Jersey: The Blackburn Press.

Simpfendorfer, C. A. & Kyne, P. M. (2009). Limited potential to recover from overfishing raises concerns for deep-sea sharks, rays and chimaeras. *Environmental Conservation* **36**, 97–103. doi:10.1017/S0376892909990191

Smith, C. L. (1997). National Audubon Society field guide to tropical marine fishes of the Caribbean, the Gulf of Mexico, Florida, the Bahamas, and Bermuda. New York: Alfred A. Knopf, Inc. 720 pp.

Smith, W. D., Cailliet, G. M. & Melendez, E. M. (2007). Maturity and growth characteristics of a commercially exploited stingray, *Dasyatis dipterura*. *Australian Journal of Marine and Freshwater Research* **58**, 54–66.

Snelson, F. F. Jr, Williams-Hooper, S. E. & Schmid, T. H. (1981). Notes on the occurrence, distribution and biology of elasmobranch fishes in the Indian river lagoon system, Florida. *Estuaries* **4**, 110–120.

Stehmann, M. (1974). *Pteroplatea binotata* Lunel, 1879, ein vergessenes Juniorsynonym von *Gymnura altavela* (Linnaeus, 1758) (Pisces, Batoidei, Gymnuridae). *Revue Suisse de Zoologie* **81**, 83–93.

Villavicencio-Garayzar, C. J. (1993). Notas sobre *Gymnura marmorata* (Cooper) (Pisces: Dasyatidae) en Bah´ıa Almejas, B.C.S., M´exico. *Revista de Investigaion Cientifica de la Universidad Autonoma de Baja California Sur* **5**, 63–67.

Vooren, C. M., Piercy, A. N., Snelson, F. F. Jr., Grubbs, R. D., Notarbartolo di Sciara, G. & Serena, S. (2007). *Gymnura altavela*. The IUCN Red List of Threatened Species 2007:

e.T63153A12624290. http://dx.doi.org/10.2305/IUCN.UK.2007.RLTS.T63153A12624290.en. Accessed on 07 February 2017

Walker, P. A. & Hessen, H. J. L. (1996). Long-term changes in ray populations in the North Sea. *ICES Journal of Marine Science* **53**, 1085–1093.

Walker, P. A. & Hislop, J. R. G. (1998). Sensitive skates or resilient rays? Spatial and temporal shifts in ray species composition in the central and north-western North Sea between 1930 and the present day. *ICES Journal of Marine Science* **55**, 392–402.

Wallace, J. H. (1967). The batoid fishes of the east coast of southern Africa. *Investigational Report of the Oceanographic Research Institute (Durban)* **16**, 1–56.

Walls, R. H. L., Vacchi, M., Notarbartolo di Sciara, G., Serena, F. & Dulvy, N. K. (2016). *Gymnura altavela*. The IUCN Red List of Threatened Species 2016: e.T63153A16527909. Accessed 07 February 2017

White, W. T. & Dharmadi (2007). Species and size compositions and reproductive biology of rays (Chondrichthyes, Batoidea) caught in target and non-target fisheries in eastern Indonesia. *Journal of Fish Biology* **70**, 1809–1837.

Wigley, S. E., McBride, H. M. & McHugh, N. J. (2003). Length-weight relationships for 74 fish species collected during NEFSC Research Vessel Bottom Trawl Surveys, 1992-99. NOAA Tech Memo NMFS NE 171, 26 p.

Wood-Mason, J. & Alcock, A. (1891). On the uterine villiform papillae of *Pteroplatea micrura* and their relation to their embryo. Proceedings of the Royal Society of London **49**, 359–367.

Wourms, J. P. (1977). Reproduction and development in chondrichthyan fishes. *American Zoologist* **17**, 379–410.

Wourms, J. P. (1981). Viviparity: The maternal-fetal relationship in fishes. *American Zoologist* **21**, 473 – 515.

Yokota, L. & Lessa, R.P. (2006) A nursery area for sharks and rays in Northeastern Brazil. *Environmental Biology of Fishes*, 75, 349–360.

Yokota, L. & Lessa, R. P. (2007). Reproductive biology of three ray species: *Gymnura micrura* (Bloch & Schneider, 1801), *Dasyatis guttata* (Bloch & Schneider, 1801) and *Dasyatis mariana* (Gomes, Rosa & Gadig, 2000) caught by artisanal fisheries in Northeastern Brazil. *Cahiers de Biologie Marine* **48**, 249–247.

Yokota, L., Goitein, R., Gianeti, M. D., Lessa, R. T. P. (2012). Reproductive biology of the smooth butterfly ray *Gymnura micrura*. *Journal of Fish Biology* **81**, 1315–1326.

Zhu, L., Li, L. & Liang, Z. (2009). Comparison of six statistical approaches in the selection of appropriate fish growth models. *Chinese Journal of Oceanology and Limnology* **27**, 457–467.

Zuur, A. F., Ieno, E., N., Walker, N. J., Saveliev, A. A., & Smith, G. M. (2009). Mixed effects models and extensions in ecology with R. New York: Springer.

Tables

TABLE I. Institutions and surveys that contributed specimens to this study.

Institution	Survey	Region	Stratum	Season
NOAA NMFS Northeast Fisheries Science Center, Woods Hole, MA	Multispecies Bottom Trawl Survey	NW to Mid-Atlantic Bight	Offshore 15+ m	Spring Winter
Virginia Institute of Marine Science Multispecies Research Group, Gloucester Point, VA	Northeast Area Monitoring & Assessment Program (NEAMAP) Bottom Trawl Survey	Mid-Atlantic Bight	Coastal 7–27 m	Spring Fall
Virginia Institute of Marine Science Multispecies Research Group, Gloucester Point, VA	Chesapeake Bay Multispecies Monitoring & Assessment Program (ChesMMAP) Bottom Trawl Survey	Chesapeake Bay	Bay, estuary 7–27 m	Year-round
Virginia Institute of Marine Science Fisheries Department, Gloucester Point, VA	Juvenile Fish & Blue Crab Trawl Survey	Chesapeake Bay	Bay, estuary 1–30 m	Year-round
South Carolina Department of Natural Resources, Marine Resources Research Institute, Charleston, SC	Southeast Area Monitoring & Assessment Program (SEAMAP) Bottom Trawl Survey, Sea Turtle Trawl Survey	South Atlantic Bight	Coastal 4–12 m	Spring Summer Fall
University of North Florida, Shark Biology Program, Jacksonville, FL	Cooperative Atlantic States Shark Pupping & Nursery (COASTSPAN) Bottom Longline Survey	FL Atlantic Coast	Bay, estuary <1-15 m	Spring Summer Fall
Florida Fish and Wildlife Research Institute, Fisheries Independent Monitoring Program, Jacksonville, FL	Bottom Trawl Survey	FL Atlantic Coast	9–110 m	Spring Summer Fall
Florida State University Coastal and Marine Laboratory, St. Teresa, FL	Gulf of Mexico Shark Pupping & Nursery (GULFSPAN) Bottom Longline & Gillnet Survey	FL Gulf of Mexico Coast	Coastal 1–18 m	Spring Summer Fall

University of South Alabama, Dauphin Island Marine Laboratory, Dauphin Island, AL	Bottom Trawl Sampling	AL Gulf of Mexico Coast	Coastal 3–30 m	Year-round
Texas Parks & Wildlife Department, Coastal Fisheries Division, Corpus Christi, TX	Bottom Trawl Survey	TX Gulf of Mexico Coast	Coastal bays, estuaries 0–90 nm offshore	Year-round

TABLE II. Gymnurid maturity stage classification scheme (adapted from Yokota *et al*., 2012). Size at birth (W_{DB}) estimated from largest embryo and smallest free-swimming individual disk width. *Estimated from largest ovarian follicle from immature specimen.

Maturity stage	Male	Female
W_{DB}		*G. altavela* WNA 300–427 mm *G. micrura* WNA 205–239 mm *G. micrura* GOM 120–242 mm
Immature	Flaccid clasper, with length not exceeding posterior margin of pelvic fin; testes homogeneous or with small translucent vesicles on ventral surface; seminal vesicle undifferentiated, thread-like.	Ovary homogeneous or exhibiting small follicles without vitellogenic activity; oviducal gland not evident; thread-like uterus with width equal to oviduct or slightly larger.
Maturing	Clasper becoming rigid, length may exceed posterior margin of pelvic fin; glans becoming structured; testes with some lobes evident; seminal vesicle beginning to enlarge.	Ovary with vitellogenic follicle diameters* <13.1 mm (*G. altavela*) and 5.1 mm (*G. micrura*); oviducal gland evident; uterus distinguishable, larger than oviduct.
Mature	Clasper fully developed and calcified (may be worn), length far exceeding posterior margin of pelvic fin, distal region fully structured and may be open in fresh specimens; testis large and with lobes evident and salient; seminal vesicles large and differentiated, may or may not exhibit sperm.	Ovary with vitellogenic follicle diameters* ≥13.2 mm (*G. altavela*) and 5.2 mm (*G. micrura*); oviducal gland fully differentiated; uterus fully developed and differentiated, vascularized and larger than oviduct, may or may not exhibit eggs or embryos.

TABLE III. Disk length (L_D, mm), disk width (W_D, mm), and total wet mass (M, kg) of *Gymnura altavela* and *G. micrura* specimens collected for this study.

		Immature			Mature		
		Mean ± S.E.	Range	n	Mean ± S.E.	Range	n
G. altavela				Western N Atlantic			
	L_{DF}	325.27 ± 15.24	202–711	33	923.42 ± 33.03	775–1185	12
	W_{DF}	646.33 ± 23.02	427–1397	43	1751.00 ± 50.83	1178–2150	19
	M_{TF}	2.64 ± 0.56	0.56–24.23	43	57.33 ± 3.97	24.00–80.26	16
	L_{DM}	299.90 ± 5.86	258–409	30	613.75 ± 11.04	535–721	20
	W_{DM}	625.42 ± 15.20	529–970	33	1214.53 ± 16.91	1021–1365	30
	M_{TM}	2.28 ± 0.40	1.14–14.30	33	16.71 ± 0.74	10.39–25.50	29
G. micrura							
	L_{DF}	209.20 ± 11.72	121–340	35	429.21 ± 7.98	287–596	68
	W_{DF}	367.35 ± 12.47	220–595	65	731.53 ± 10.63	506–1029	95
	M_{TF}	0.54 ± 0.06	0.09–2.02	65	4.39 ± 0.20	1.25–10.70	95
	L_{DM}	179.12 ± 8.27	114–270	33	267.52 ± 4.53	175–333	44
	W_{DM}	324.88 ± 7.50	205–450	66	431.14 ± 5.54	293–528	57
	M_{TM}	0.33 ± 0.03	0.07–0.87	66	0.78 ± 0.03	0.24–1.65	56
G. micrura				Gulf of Mexico			
	L_{DF}	203.13 ± 17.44	145–302	8	368.55 ± 13.39	291–490	20
	W_{DF}	364.25 ± 31.13	260–544	8	645.18 ± 15.33	412–856	44
	M_{TF}	0.50 ± 0.16	0.14–1.49	8	2.80 ± 0.20	1.07–5.55	37
	L_{DM}	162.00 ± 10.99	135–222	7	233.38 ± 3.65	166–285	40
	W_{DM}	277.38 ± 14.13	242–364	8	381.45 ± 4.56	294–459	47
	M_{TM}	0.15 ± 0.02	0.09–0.23	7	0.51 ± 0.02	0.32–0.90	30

TABLE IV. (a) Mass-at-disk width model (M_{MW}) results; (b) gonadosomatic index model (M_{IG}) and hepatosomatic index model (M_{IH}) results from beta regression analysis of the effect of month and sex; (c) oocyte diameter linear mixed effects model (M_{DO}) results of the relationship between mean oocyte diameter (mm) and month with covariates for individual female and associated W_D in *Gymnura altavela* and *G. micrura*. Sample sizes are provided in parentheses (n_{Sex}), and the selected models are indicated in bold.

		Model	LL	Covariates	No. of parameters	AIC_c	ΔAIC_c
(a)	Western N Atlantic			M-W_D Regression			
	G. altavela (58_F, 63_M)	**$M1_{MW}$**	**-40.69**	**None**	**4**	**-34.49**	**0**
		$M2_{MW}$	-41.54	α_{Sex}	5	-33.19	1.29
		$M3_{MW}$	-41.62	β_{Sex}	5	-33.27	1.22
		$M4_{MW}$	-42.06	$\alpha_{Sex}\,\beta_{Sex}$	6	-31.54	2.95
	G. micrura (166_F, 128_M)	$M1_{MW}$	419.39	None	4	413.3	14.06
		$M2_{MW}$	434.99	α_{Sex}	5	426.85	0.51
		$M3_{MW}$	**435.5**	**β_{Sex}**	**5**	**427.36**	**0**
		$M4_{MW}$	436.92	$\alpha_{Sex}\,\beta_{Sex}$	6	426.71	0.65
	Gulf of Mexico						
	G. micrura (28_F, 38_M)	**$M1_{MW}$**	**-40.69**	**None**	**4**	**110.19**	**0**
		$M2_{MW}$	-41.54	α_{Sex}	5	109.12	1.07
		$M3_{MW}$	-41.62	β_{Sex}	5	109.08	1.12
		$M4_{MW}$	-42.06	$\alpha_{Sex}\,\beta_{Sex}$	6	107.1	3.09
(b)	Western N Atlantic			I_G, I_H Beta regression			
	G. altavela (39_F, 26_M)	**$M1_{IG}$**	**-425.11**	***Sex***	**4**	**-415.29**	**0**
		$M2_{IG}$	-408.99	None	3	-401.81	13.48
	(12_F, 27_M)	$M1_{IH}$	-266.08	*Sex*	4	-256.26	0.6
		$M2_{IH}$	**-264.04**	**None**	**3**	**-256.86**	**0**
	G. micrura (81_F, 27_M)	$M1_{IG}$	1171.51	*Sex*	9	1151.24	1.72
		$M2_{IG}$	**1170.8**	**None**	**8**	**1152.96**	**0**
	(83_F, 37_M)	$M1_{IH}$	-727.25	*Sex*	9	-707.23	2.18
		$M2_{IH}$	**-727.05**	**None**	**8**	**-709.41**	**0**
	Gulf of Mexico						
	G. micrura (27_F, 20_M)	**$M1_{IG}$**	**-568.97**	***Sex***	**5**	**-556.87**	**0**
		$M2_{IG}$	-562.38	None	4	-552.92	3.95
	(35_F, 23_M)	**$M1_{IH}$**	**-451.81**	***Sex***	**5**	**-440.16**	**0**

		$M2_{IH}$	-443.16	None	4	-434.01	6.15
(c)	Western N Atlantic			D_O Linear mixed effects			
	G. altavela (81_F)	$M1_{Do}$	455.77	*Ind* W_D	7	471.77	2.38
		$M2_{Do}$	**455.86**	***Ind***	**6**	**469.39**	**0**
	G. micrura (218_F)	$M1_{Do}$	834.04	*Ind* W_D	9	853.1	2.12
		$M2_{Do}$	**834.12**	***Ind***	**8**	**850.9**	**0**
	Gulf of Mexico						
	G. micrura (176_F)	$M1_{Do}$	88.17	*Ind* W_D	6	100.84	0.5
		$M2_{Do}$	**89.84**	***Ind***	**5**	**100.34**	**0**

LL is negative log-likelihood.

Table V. Parameter estimates, standard errors (S.E.), predicted means, and lower and upper confidence limits (C.L.) for the selected (a) mass-at-disk width (*MW*) models; (b) monthly gonadosomatic index (I_G) and hepatosomatic index (I_H) beta regression models; (c) monthly oocyte diameter linear mixed effects models (M_{DO}) for *Gymnura altavela* and *G. micrura*. Model estimates and S.E. for $MW\alpha$ models are expressed as 10^{-10}; S.E. for $MW\beta$ are expressed as 10^{-1}; predicted mean and C.L. for I_G are expressed as 10^{-2}.

		Parameter	Estimate	S.E.	Predicted mean	C.L.
(a)	Western N Atlantic			M-W_D Regression		
	G. altavela	$MW_{1\alpha}$	27.80	8.34		
		$MW_{1\beta}$	3.17	0.45		
	G. micrura	$MW_{3\alpha}$	12.00	1.52		
		$MW_{3\beta M}$	3.34	0.22		
		$MW_{3\beta F}$	3.38	0.03		
	Gulf of Mexico					
	G. micrura	$MW_{1\alpha}$	30.48	7.74		
		$MW_{1\beta}$	3.18	0.41		
(b)	Western N Atlantic			I_G, I_H Beta regression		
	G. altavela	I_{G1FSep}	-5.07	0.17	0.44	0.35–0.53
		I_{G1FOct}	-0.02	0.16	0.30	0.25–0.35
		I_{G1MSep}	-5.43	0.09	0.63	0.56–0.69
		I_{G1MOct}	-5.81	0.07	0.43	0.38–0.48
		I_{H2Sep}	-3.12	0.05	4.24	3.88–4.60
		I_{H2Oct}	-3.33	0.07	3.47	3.11–3.82
	G. micrura	I_{G2May}	-5.97	0.12	0.25	0.21–0.30
		I_{G2Jun}	-5.89	0.18	0.28	0.26–0.29
		I_{G2Jul}	-5.29	0.16	0.50	0.32–0.68
		I_{G2Aug}	-5.71	0.17	0.33	0.22–0.44
		I_{G2Sep}	-5.93	0.18	0.38	0.24–0.53
		I_{G2Oct}	-5.78	0.13	0.31	0.28–0.33
		I_{G2Nov}	-5.42	0.17	0.44	0.31–0.57
		I_{H2May}	-2.87	0.08	5.38	4.66–6.09
		I_{H2Jun}	-3.31	0.14	3.53	3.24–3.82
		I_{H2Jul}	-3.44	0.16	3.10	2.38–3.82
		I_{H2Aug}	-3.73	0.17	2.35	1.87–2.84
		I_{H2Sep}	-3.50	0.18	2.94	2.49–3.38
		I_{H2Oct}	-3.09	0.09	4.36	4.04–4.68

		I_{H2Nov}	-3.13	0.15	4.18	3.05–5.31
	Gulf of Mexico					
	G. micrura	I_{G1FMay}	-6.61	0.25	0.19	0.15–0.24
		I_{G1FJul}	-6.93	0.27	0.14	0.12–0.16
		I_{G1FOct}	-6.63	0.42	0.19	0.18–0.20
		I_{H1FMay}	-3.65	0.11	3.00	2.78–3.23
		I_{H1FJul}	-3.67	0.12	2.50	2.25–2.74
		I_{H1FOct}	-3.83	0.22	2.12	2.08–2.15
		I_{G1MMay}	-6.24	0.11	0.14	0.06–0.21
		I_{G1MJul}	-6.56	0.13	0.10	0.07–0.13
		I_{G1MOct}	-6.26	0.29	0.13	0.08–0.19
		I_{H1MMay}	-3.47	0.05	2.54	2.26–2.81
		I_{H1MJul}	-3.67	0.06	2.11	1.97–2.24
		I_{H1MOct}	-3.83	0.16	1.79	1.57–2.00
(c)	Western N Atlantic		D_O Linear mixed effects			
	G. altavela	D_{O2Mar}	24.16	5.15	24.16	14.07–34.25
		D_{O2Aug}	7.70	7.37	7.70	-2.64–18.04
		D_{O2Sep}	18.71	5.76	18.71	13.66–23.75
		D_{O2Oct}	11.25	5.38	11.25	8.2–14.29
	G. micrura	D_{O2May}	5.10	1.59	5.10	0.86–9.34
		D_{O2Jun}	9.63	1.69	9.63	6.55–12.72
		D_{O2Aug}	4.21	1.74	4.21	1.02–7.42
		D_{O2Sep}	14.96	2.25	14.96	10.72–19.20
		D_{O2Oct}	4.00	1.61	4.00	1.07–6.92
		D_{O2Nov}	2.78	2.25	2.78	-1.46–7.02
	Gulf of Mexico					
	G. micrura	D_{O2May}	1.04	0.12	1.04	0.97–5.40
		D_{O2Jul}	1.01	0.15	1.01	0.95–5.22
		D_{O2Oct}	1.33	0.32	1.33	1.62–6.88

Figures

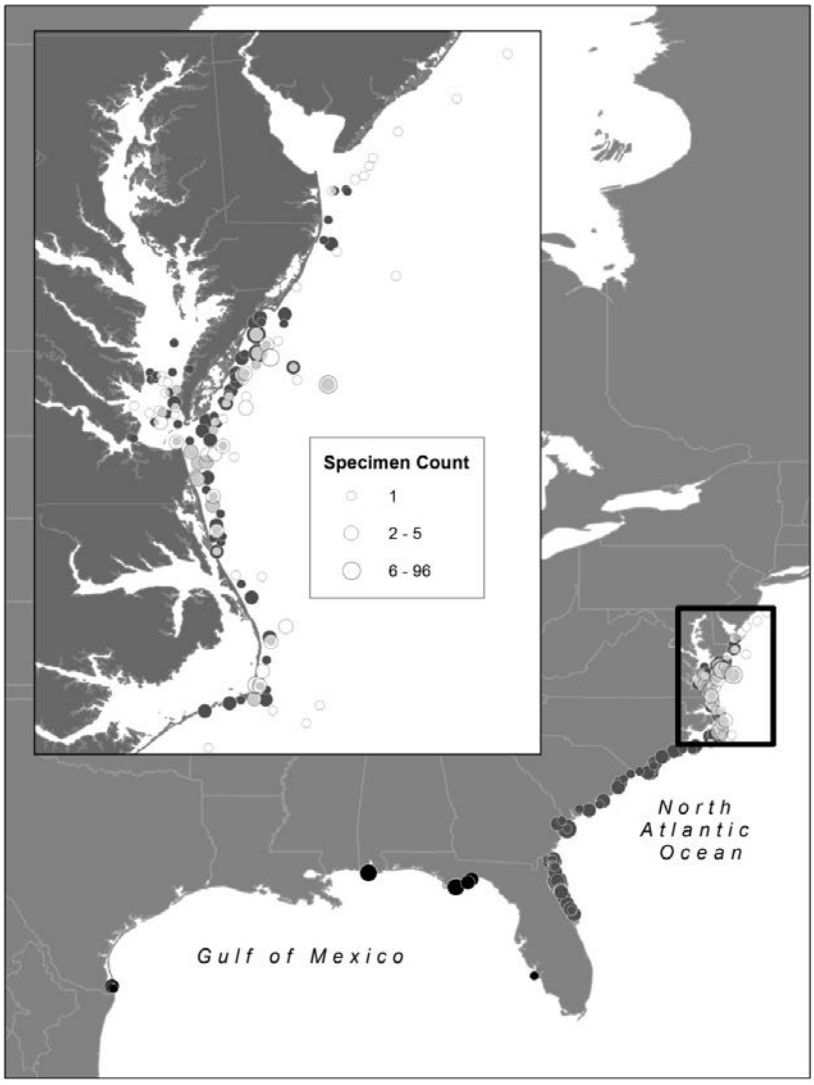


FIG. 1. Map depicting the distribution of *Gymnura altavela* (North Atlantic ● *n* = 127) and *G. micrura* (North Atlantic ● *n* = 295; Gulf of Mexico ● *n* = 120) collected between 2012 and 2016.

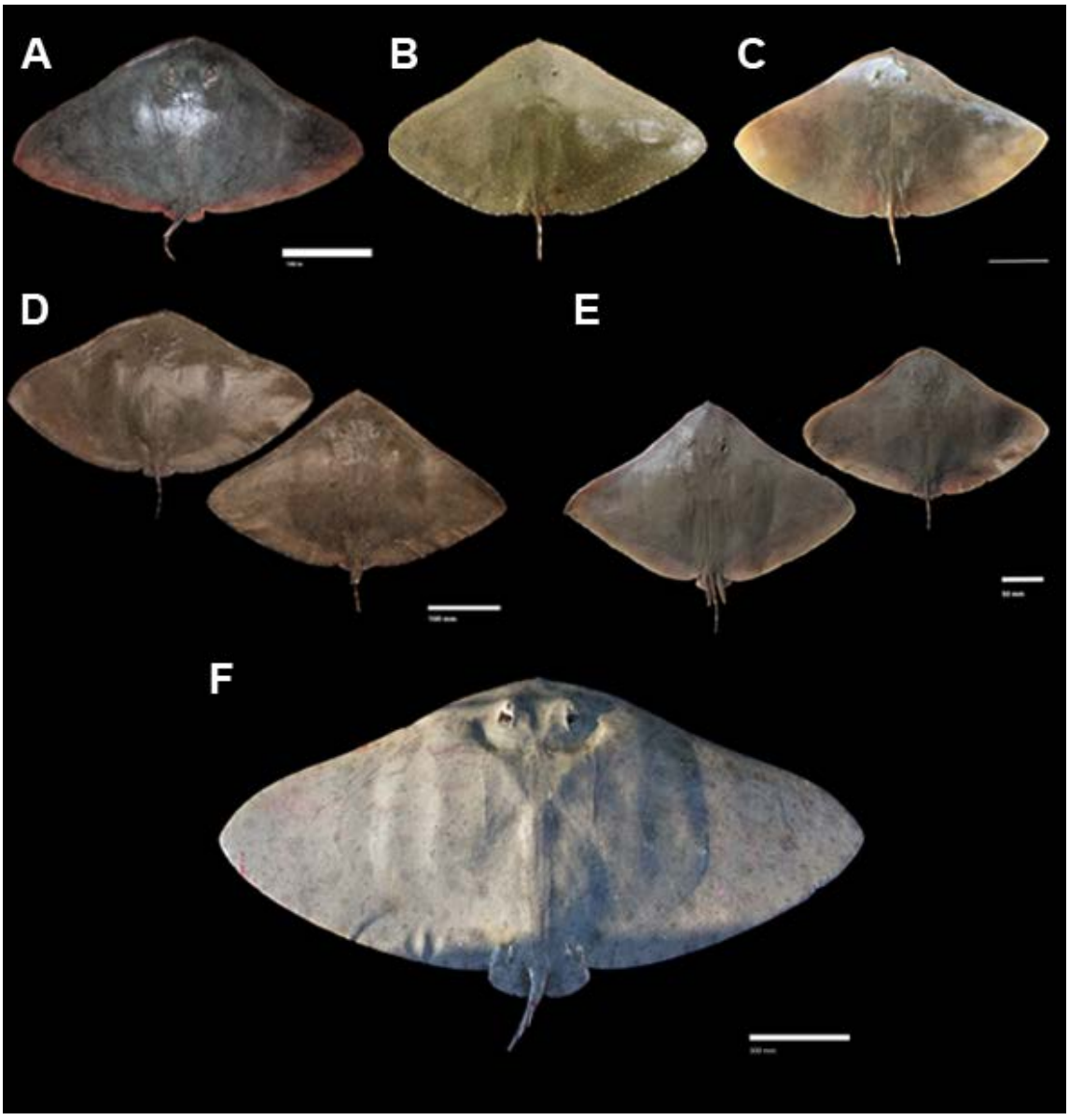


FIG. 2. Life stages of western Atlantic *Gymnura*: young-of-year (a) *G. altavela*, (b) western North Atlantic *G. micrura*, and (c) Gulf of Mexico *G. micrura*; sexual dimorphism between (d) juvenile female and adult male western North Atlantic *G. micrura*; ontogenetic comparison of morphology between (e) adult and juvenile male Gulf of Mexico *G. micrura*; (f) adult female *G. altavela.*

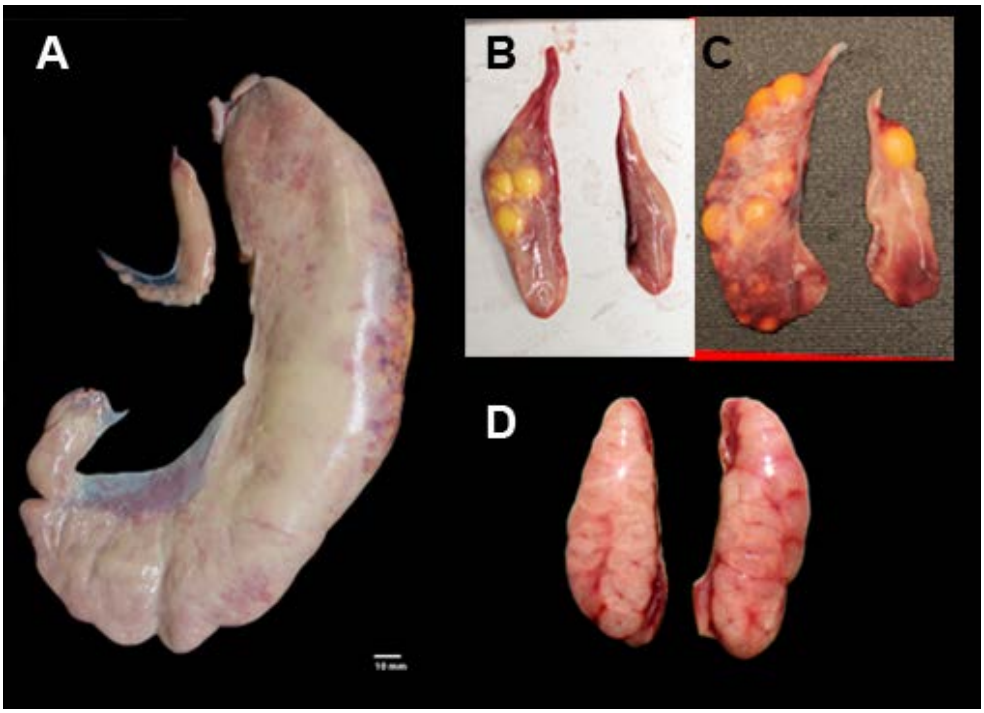


FIG. 3. Gonads from mature western Atlantic *Gymnura*: (a) right and left ovaries of *Gymnura altavela*, (b) left and right ovaries of *G. micrura*, (c) left and right ovaries of *G. micrura*, with enlarged oocyte in the right ovary, (d) similarly developed left and right testes of *G. micrura*.

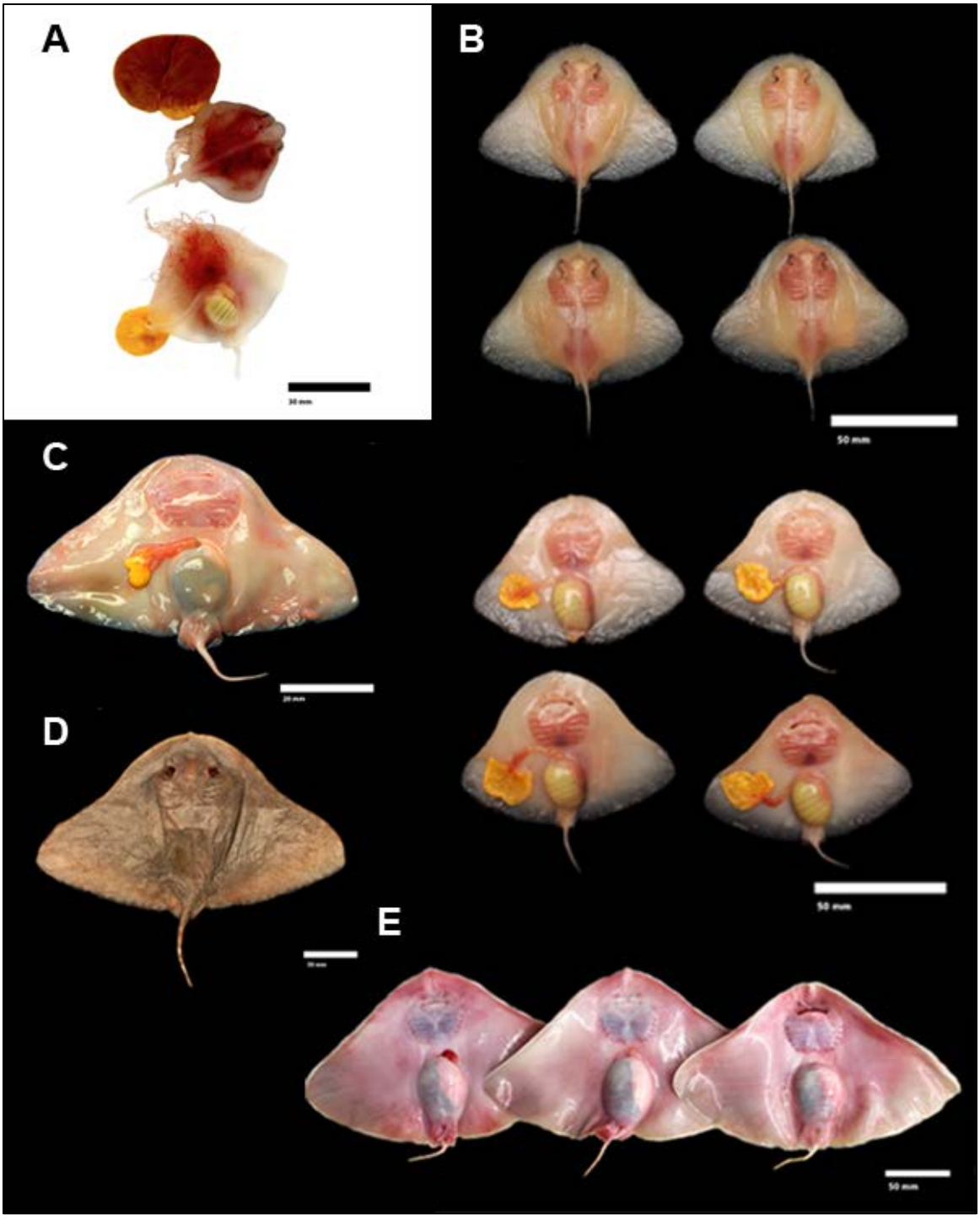


FIG. 4. Embryonic development stages: (a) dorsal and ventral view of early-stage *Gymnura micrura*, (b) dorsal and ventral view of mid-stage *G. micrura*, (c) ventral view of mid-stage *G. altavela*, and (d) dorsal and (e) ventral view of late-stage *G. altavela*.

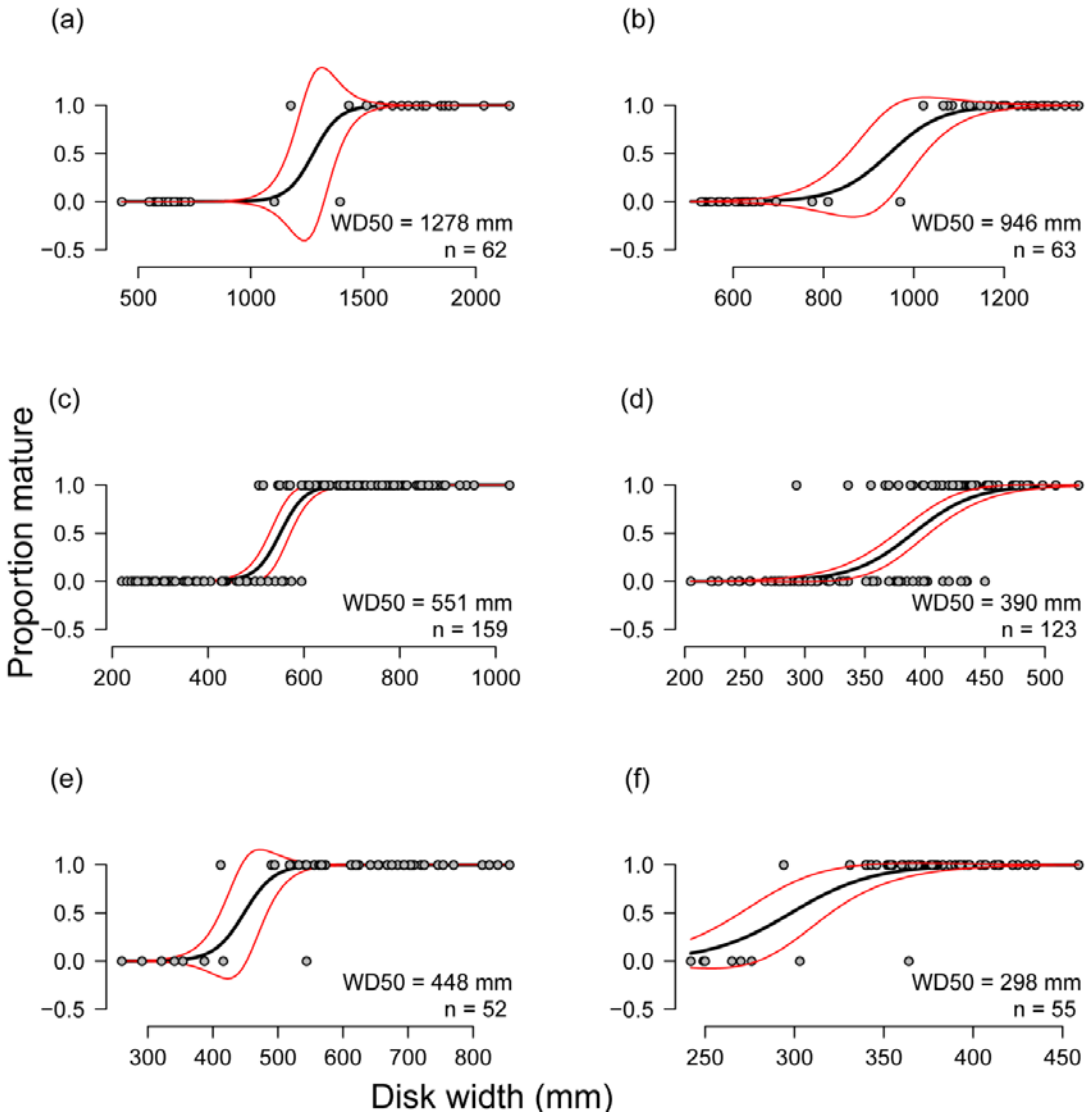


FIG. 5. Maturity ogives for (a) female (n = 43 immature, 19 mature) and (b) male (n = 33 immature, n = 30 mature) *Gymnura altavela*, (c) female (n = 65 immature, 94 mature) and (d) male (n = 66 immature, n = 57 mature) western North Atlantic *G. micrura*, and (e) female (n = 8 immature, 44 mature) and (f) male (n = 8 immature, n = 47 mature) northern Gulf of Mexico *G. micrura*.

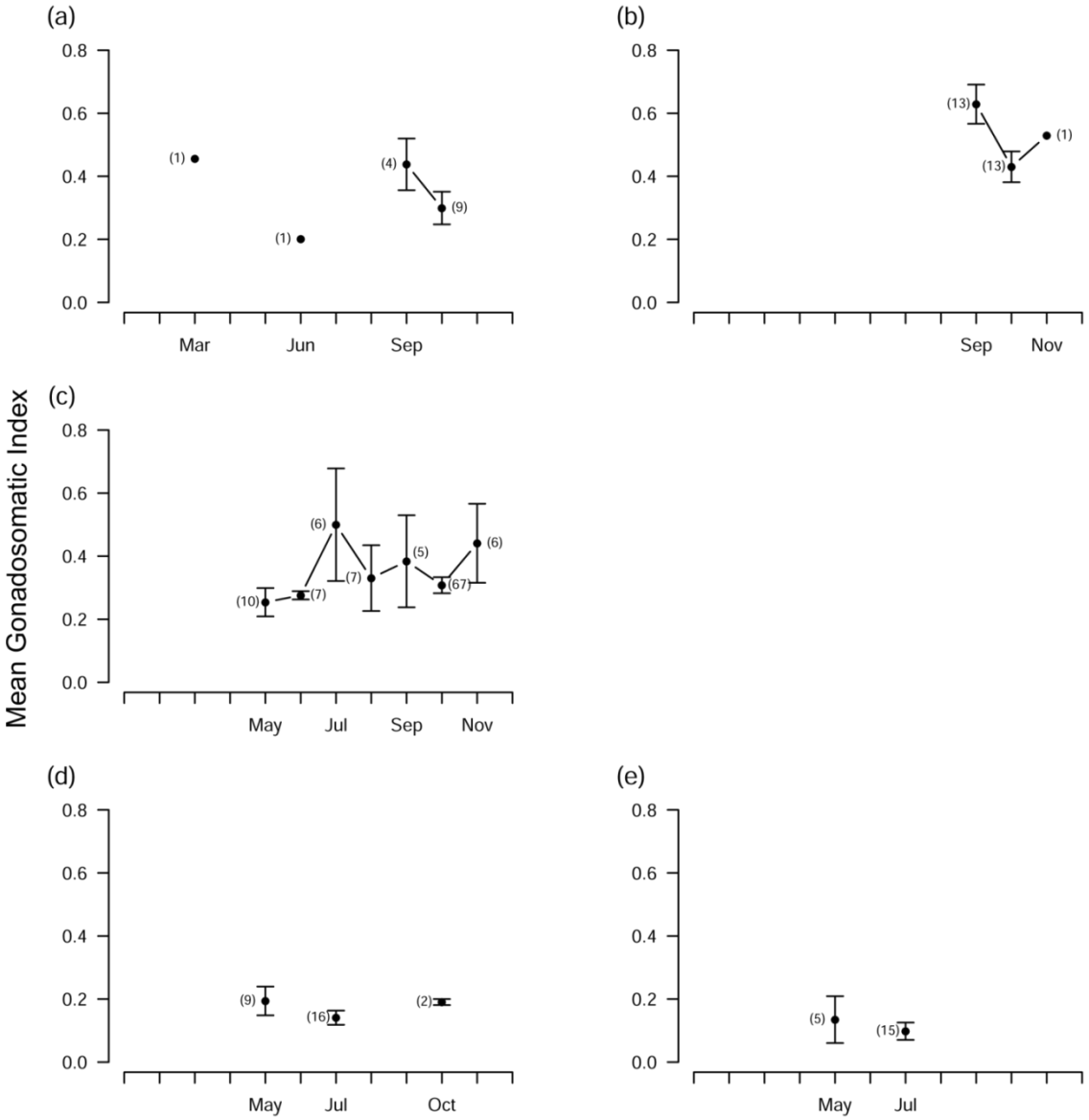


FIG. 6. Monthly mean gonadosomatic index (I_G) of (a) female (n = 15 mature) and (b) male (n = 27 mature) *Gymnura altavela*, (c) female (n = 114 mature) and male (n = 87 mature) western North Atlantic *G. micrura* (sex data are pooled), and (d) female (n = 27 mature) and (e) male (n = 20 mature) northern Gulf of Mexico *G. micrura*. Error bars are 95% upper and lower confidence limits, and parentheses indicate sample size.

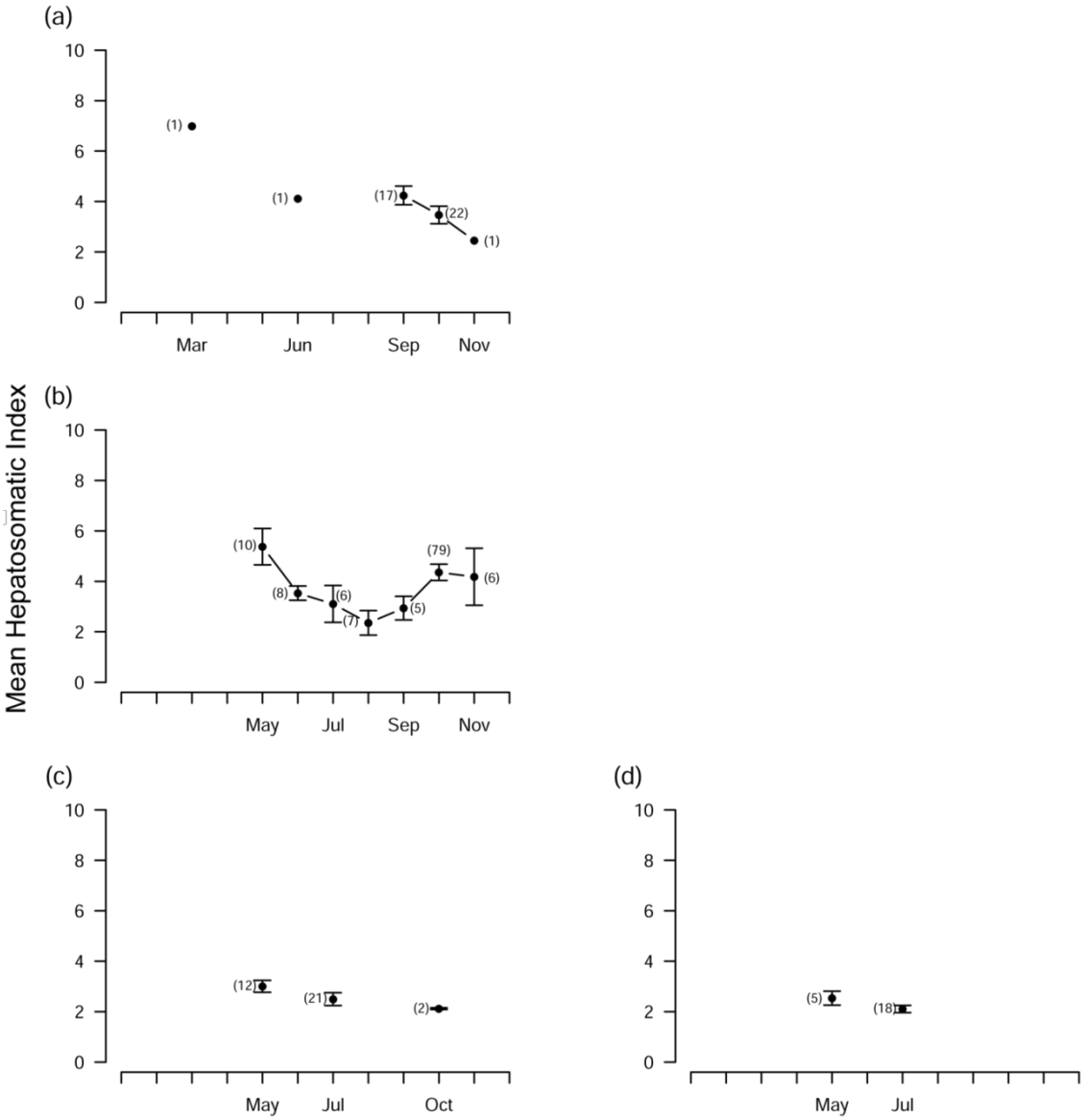


FIG. 7. Monthly mean hepatosomatic index (I_H) of (a) female (n = 19 mature) and male (n = 30 mature) *Gymnura altavela* (b) female (n = 83 mature) and male (n = 38 mature) western North Atlantic *G. micrura*, and (c) female (n = 35 mature) and (d) male (n = 23 mature) northern Gulf of Mexico *G. micrura*. Error bars are 95% upper and lower confidence limits, and parentheses indicate sample size.

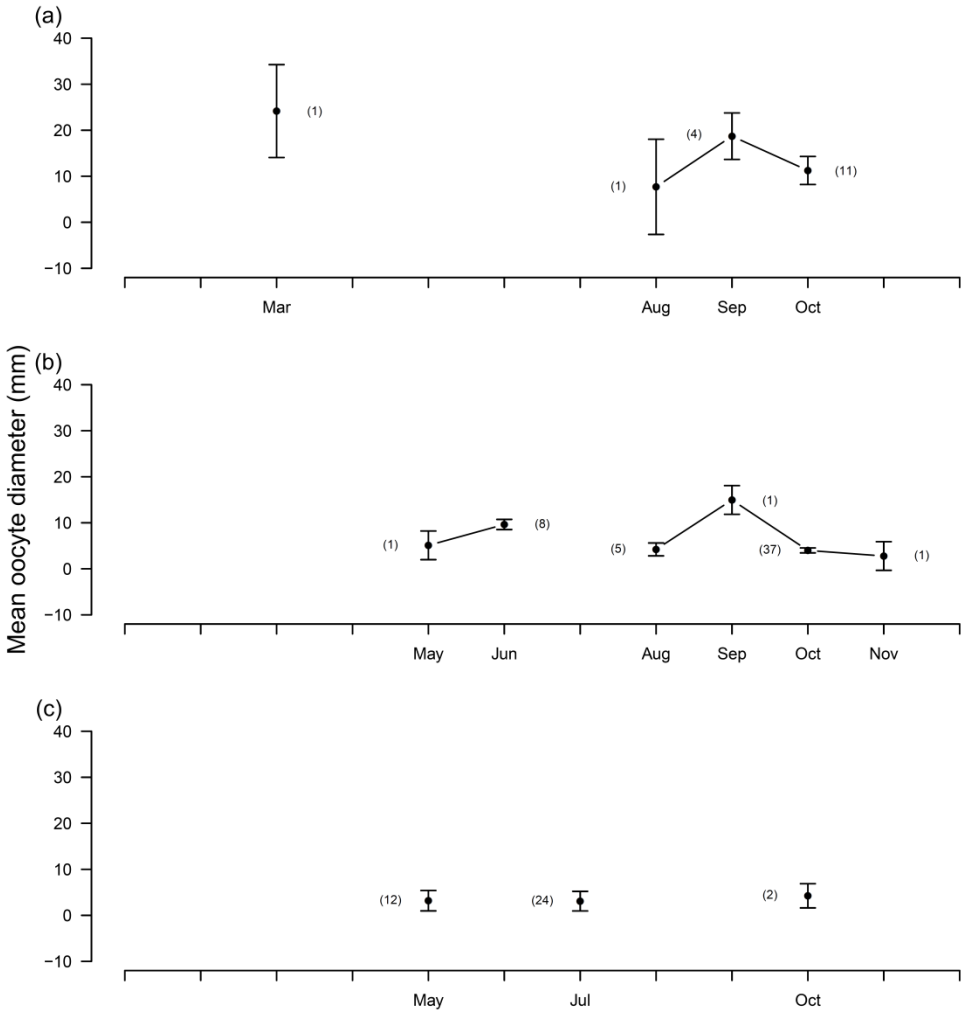


FIG. 8. Monthly variation in linear mixed effects model predicted D_O of (a) *Gymnura altavela*, (b) western North Atlantic *G. micrura*, and (c) Gulf of Mexico *G. micrura*, with individual female treated as a random effect. Error bars are upper and lower 95% confidence intervals, and the number of individual females is indicated in parentheses.

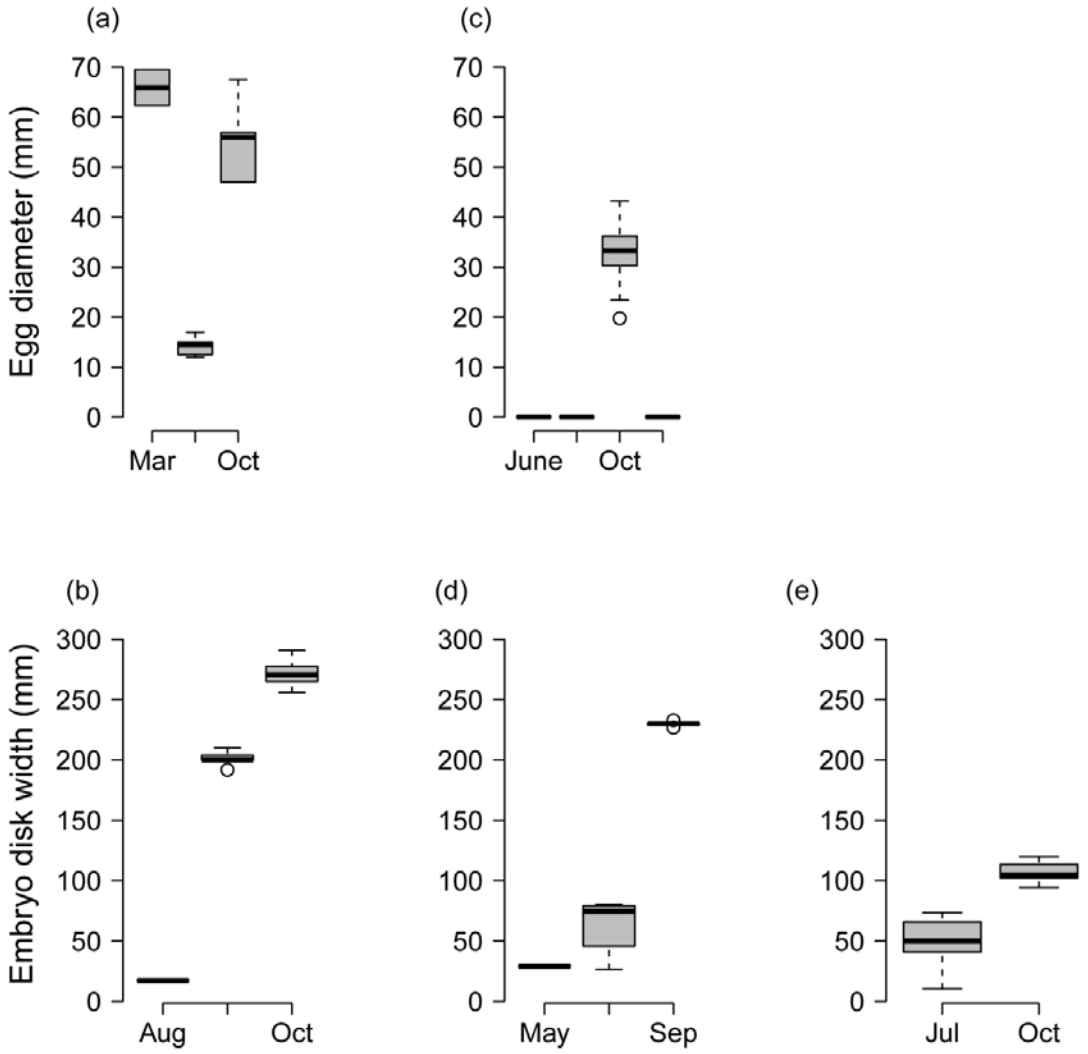


FIG. 9. Monthly egg (a, c) and embryo (b, d, e) sizes observed in western North Atlantic (a, b) *Gymnura altavela* (a, b) and *G. micrura* (c, d), and Gulf of Mexico *G. micrura* (e). Boxes represent the upper and lower quartiles, and the black line indicates the median; error bars are the range, and outliers are indicated by open circles. In panel c, months when eggs were observed but not measurable are represented at 0 mm.

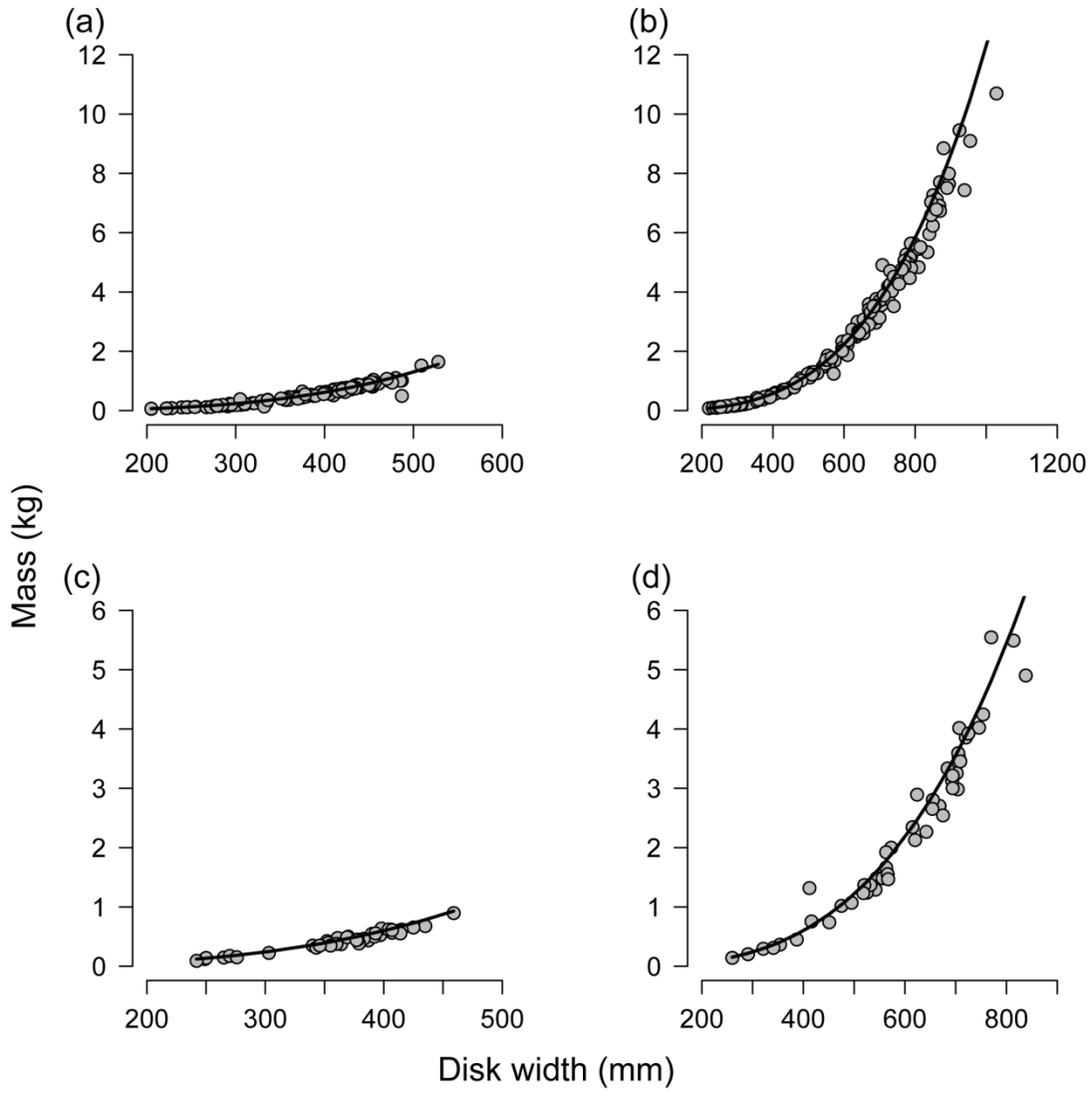


FIG. 10. Weight-at-disk width relationships for (a) male (n = 128) and (b) female (n = 166) western North Atlantic *Gymnura micrura*, and (c) male (n = 38) and (d) female (n = 48) Gulf of Mexico *G. micrura*.

CHAPTER 4

Morphological and Genetic Analyses of *Gymnura micrura* (Myliobatiformes: Gymnuridae) from the Western North Atlantic Ocean Reveal Two New Species of Butterfly Ray

Abstract

Batoid fishes (skates, rays, and guitarfishes) are among the most threatened and least understood chondrichthyan species worldwide due to their large body size, conservative life-history characteristics, and predominantly coastal distributions where fishing and habitat degradation threaten the stability of populations. Many taxa are in need of taxonomic re-examination and species-specific population assessment. The Smooth Butterfly Ray (*Gymnura micrura*, Bloch & Schneider 1801) is widely distributed throughout the Atlantic Ocean, and considered 'Data Deficient' by the International Union for Conservation of Nature and Natural Resources. In shallow coastal regions, *G. micrura* is common bycatch in demersal trawl fisheries due to habitat overlap with commercially valuable marine resources; however incidental catch data and life history parameter estimates are lacking for this species in U.S. waters. Furthermore, the identification of *G. micrura* has been complicated by poorly described morphological variation. Previous descriptions do not fully account for sexual dimorphism or other variation in morphology and life history that are observed throughout its range, and type material is not available to evaluate the taxonomic status of the species. Resolving the taxonomy of *G. micrura* is essential for the assessment of populations and their vulnerability to extinction. In the present study, we revise the taxonomy of *G. micrura* based on life history, morphology, and molecular traits. Comparative morphometric and mitochondrial (ND2) and nuclear (RAG-1) genetic analyses revealed multiple distinct species in the western Atlantic. A re-description of *G. micrura* is presented, and two new species are described from the western Atlantic and the northern Gulf of Mexico. A key to the identification of species in the region is also provided. The conservation

and sustainable management of Atlantic Gymnuridae requires careful consideration of species-specific taxonomy and biology to accurately assess the status of contemporary populations, and to document and maintain the true biodiversity of this taxon.

Key words: Batoidea; morphometric analyses; CCA; nasal curtain length; color pattern; ND2; RAG-1

Introduction

The Butterfly Rays (Myliobatiformes: Gymnuridae: *Gymnura* van Hasselt, 1823) comprise at least 10 species that are distributed worldwide in tropical and warm temperate seas (Compagno *et al*. 1989; Last & Stevens 2009; McEachran & de Carvalho 2002; Eschmeyer & Fong 2015; Last *et al*. 2016; Weigmann 2016). Butterfly Rays are commonly associated with shallow marine and brackish waters, and prefer sandy and muddy substrates (Murdy *et al*. 2013). Members of the genus *Gymnura* are distinguished from other rays by a rhomboid and dorso-ventrally compressed body shape, in which the width of the disk is approximately twice the disk length, and a short tail that often has light and dark crossbars and one or more serrated spines, in some species (Compagno & Last 1999; Last & Stevens 2009).

In the US Atlantic Ocean and Gulf of Mexico, two gymnurid species are currently recognized: the Spiny Butterfly Ray, *Gymnura altavela* (Linnaeus 1758), and the Smooth Butterfly Ray, *G. micrura* (Bloch & Schneider 1801). Both species are described as also occurring in the eastern Atlantic, and a third species, *G. natalensis* (Gilchrist & Thompson 1911) can be found off the coast of southwest Africa (Weigmann 2016). The validity of a fourth Atlantic species, *G. hirundo* (Lowe 1843), requires further taxonomic evaluation, and may represent a junior synonym of *G. altavela* (Weigmann 2016); this taxon was not included in the family account by Yokota *et al*. (2016). In the western Atlantic, the range of distribution of *G. micrura* extends from the northeastern US and Gulf of Mexico to Brazil (Last *et al*. 2016), while *G. altavela* can be found from the northeastern US and Gulf of Mexico to northern Argentina (Robins & Ray 1986; McEachran & Séret 1990; McEachran & de Carvalho 2002). *Gymnura micrura* and *G. altavela* are

distinguished by the lack of one or more serrated tail spines and spiracular tentacles in the former, and the maximum size of *G. altavela* (> 2000 mm DW), which greatly exceeds that of *G. micrura* (< 1200 mm DW). Both species are also reported from the eastern Atlantic, although recent studies suggest these populations may represent separate, undescribed species (e.g. Naylor *et al*. 2012; Last *et al*. 2016; Weigmann 2016). Body shape and coloration are highly conserved across the genus (Jacobsen & Bennett 2009; Smith *et al*. 2009), thus tail morphology has often been used as a primary diagnostic character for species identification (Bigelow & Schroeder 1953; Murdy *et al*. 1997; Compagno & Last 1999). Original species descriptions with inadequate consideration for variation in body shape due to sexual dimorphism and ontogeny (e.g., Bigelow & Schroeder 1953; Smith *et al*. 2009) have contributed to taxonomic confusion and uncertainty in the status of many species, particularly in the absence of a holotype. Consequently, taxonomic revision of the Gymnuridae and re-descriptions of most taxa are needed (Muktha *et al*. 2016; Jacobsen & Bennett 2009; Smith *et al*. 2009).

Life history strategies of batoid fishes have been characterized by slow growth to maturation and low fecundity that increases the vulnerability of populations to depletion from overexploitation (e.g. Dulvy *et al*. 2008). Effective management and conservation of batoid populations relies on species-specific assessments, which to date have been hindered by a paucity of life-history information for nearly half of all taxa (Dulvy *et al*. 2014). Species with circumglobal distributions are in particular need of detailed reassessment, as many taxa have recently been found to belong to species complexes (White & Last 2012). Currently, *G. micrura* distributed in US waters have no commercial value, and the species is not targeted by fisheries, although incidental capture and release of the species occurs in trawl fisheries and may be substantial where densities are high. Biological information on *G. micrura,* including reproduction and diet, has been reported in several studies (Wood-Mason & Alcock 1891; Alcock 1892; Ranzi 1934; Bigelow & Schroeder 1953; Amoroso 1960; Daiber & Booth 1960; Yokota & Lessa 2007;

Yokota *et al*. 2012; Parsons 2017). However, investigations into the life history of *G. micrura* in US waters were often constrained by low sample sizes and limited spatiotemporal representativeness, resulting in fragmented and potentially inaccurate biological characterization, and designation as globally 'Data Deficient' by the International Union for Conservation of Nature and Natural Resources (IUCN) (http://www.iucnredlist.org; Grubbs & Ha 2006). Due to the absence of direct threats to the species in U.S. waters, *G. micrura* are locally considered species of Least Concern (Grubbs & Ha 2006). Recent efforts to address knowledge gaps in the life history of this species revealed geographic variation in key population parameters (e.g., size at maturity, maximum size, fecundity) and morphology (e.g., disk and tail coloration, gonad size), and suggested possible structuring in the U.S. population and the potential for cryptic speciation within the species range of distribution (Yokota & Lessa 2006, 2007; Parsons 2017). In the Gulf of Mexico, for example, the species matures at a smaller size, attains smaller maximum sizes, and produces twice the number of offspring relative to individuals from the eastern coast of the US (Parsons 2017). Specimens from these regions also display dissimilar disk coloration and patterns, raising uncertainty in the taxonomic status of *G. micrura* throughout its western North Atlantic range of distribution.

To clarify the taxonomic status of western Atlantic *G. micrura*, geographical variation in morphological and molecular (ND2 – mitochondrial NADH dehydrogenase 2; RAG-1 – nuclear recombination activating gene 1) characters from fresh and preserved specimens collected from U.S. waters were assessed and compared. This analysis incorporates the redescription of *G. micrura* and the descriptions of two new species. An updated species identification key for Gymnuridae of the western Atlantic is also included.

Materials and methods

Fresh specimens (n = 138) of *G. micrura* were collected from commercial and fishery-independent trawl surveys conducted in three geographical regions including the US coast from Maryland to Florida in the western North Atlantic (ATL), the Gulf of Mexico (GOM) coast from Florida to Texas, and the northern South America coast of Suriname (SUR; this is the type locality for *G. micrura*) between 2012 and 2016 (Fig. 1). Tissue samples were collected for genetic analysis, and morphometric data were obtained from 153 fresh individuals. Morphometric data were also collected from preserved specimens of *G. micrura* (n = 110) and eight congener taxa (*G. altavela*, *G. australis* Ramsay & Ogilby 1886, *G. crebripunctata* Peters 1869, *G. japonica* Temminck & Schlegel 1850, *G. marmorata* Cooper 1864, *G. poecilura* Shaw 1804, *G. tentaculata* Müller & Henle 1841, *G. zonura* Bleeker 1852) held in the collections of the National Museum of Natural History, Smithsonian Institution, Washington, D.C. (USNM), the Harvard University Museum of Comparative Zoology, Cambridge, MA (MCZ), the Field Museum, Chicago, IL (FMNH), the Florida Museum of Natural History, Gainesville, FL (FLMNH), and the Muséum National d'Histoire Naturelle, Paris, France (MNHN). Specimens were photographed and disk and tail coloration was recorded. Observations on disk and tail coloration from an additional 295 fresh specimens of US *G. micrura* were also documented. New specimens were deposited in the Nunnally Ichthyology Collection at the Virginia Institute of Marine Science (VIMS), the MCZ, the FLMNH, and the National Zoological Collection of Suriname (NZCS), Paramaribo, Suriname.

Terminology and abbreviations. Measurements and terminology for 23 characters in the present study follow Smith *et al.* (2009) (Fig. 2). Abbreviations for measurements include: DW: disk width; LD: disk length (tip of snout to posterior margin of disk); LB: body length (tip of snout to posterior margin of pelvic fins); LH: head length (tip of snout to apex of anterior

concavity of synarcual); LAP: anterior pectoral-fin length; LPP: posterior pectoral-fin length; LPOBS: pre-orbital snout length; WIO: inter-orbital width (anteriormost point of eyes); WIS: interspiracular width (anteriormost point of spiracles); LSV: snout to vent length (anteriormost point of vent); LSG1: snout to first gill length; DG5: 5th gill transverse distance; DG1: 1st gill transverse distance; LAPV: anterior pelvic-fin length; SP: pelvic-fin span; LPN: pre-narial length; LPOLS: pre-oral snout length; LNC: nasal curtain length; WIN: inter-narial length; WNC: nasal-curtain width; WM: mouth width; ILCL: inner left-clasper length; OLCL: outer left clasper length. Sex was determined for each specimen, and life stage (i.e., juvenile or adult) was determined based on the presence of sexually mature, calcified claspers in males, and reproductively mature ovaries or the presence of embryos in females, when possible. Measurements were made to the nearest 1.0 mm for DW, LD, LB, LAP, LPP, and LSV, and the remaining characters were measured to the nearest 0.1 mm.

Morphometric analysis. Morphometric measurements of characters from immature juveniles and mature adults of *G. micrura* were converted to proportions of DW (% DW) to evaluate relative differences in metrics between specimens collected from four geographical regions: 1) Western North Atlantic (ATL), Delaware to southeast Florida, US; 2) Gulf of Mexico (GOM), Florida Keys to southern Texas, US; 3) Suriname (SUR), Venezuela to French Guiana, South America; 4) Eastern Atlantic, Senegal to Angola.

To explore the relationship between metrics and the geographical origin of western Atlantic specimens, canonical correlation analysis (CCA) was used. CCA is a multivariate technique for direct gradient analysis (ter Braak, 1986), and for morphological analyses, can be applied to investigate how characters differ between specimens in relation to explanatory variables. Proportion metrics from females (n = 96) and males (n = 91) originating from the ATL, GOM, and the type locality (Suriname specimens only) were used for analysis and treated separately to account for sexual dimorphism. Correlation between metrics was evaluated using

the Pearson correlation coefficient, and highly correlated metrics ($\rho > 0.85$) were removed, resulting in a dataset of 10 metrics for females (WIO, WIS, LSV, DG5, DG1, SP, LNC, WIN, WNC, WM) and males (WIS, LSV, DG5, DG1, LAPV, SP, LNC, WIN, WNC, WM). Metrics were square root transformed and conspicuous outliers were removed to satisfy methodological assumptions. CCA was performed using a fully saturated model (i.e., 10 metrics), and ANOVA was used to assess statistical significance of the resulting canonical axes and the effect of region. All statistical analyses were performed in the R environment (R Development Core Team 2016).

Molecular analysis. Tissue samples collected from *G. micrura* specimens were stored in 95% EtOH for mitochondrial (ND2) and nuclear (RAG-1) DNA analysis. DNA was extracted from tissue samples using DNeasy Blood & Tissue Kits (QIAGEN, Valencia, CA). Polymerase chain reaction (PCR) amplification and Sanger sequencing was performed after testing DNA quality using a Nanodrop Spectrophotometer (ThermoFisher Scientific, Waltham, MA). Amplification of the mitochondrial ND2 locus was initially performed using primers published in Naylor *et al.* (2005, 2012), and the nuclear RAG-1 gene was amplified using previously developed primers (McDowell, unpubl. data). New primers were developed for the ND2 gene using Primer3 v 4.0 software (Koressaar & Remm 2007; Untergrasser *et al*. 2012) when established primers failed to amplify. PCR was performed on all samples and conditions were optimized for subsequent bi-directional cycle sequencing on an ABI 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA). Resulting DNA sequences were edited and forward and reverse sequences were assembled using the Sequencher 5.1 software (Gene Codes Corp. Ann Arbor, MI) with uncertainties in the chromatograms coded as ambiguities. Sequences were and aligned using the MAFFT v7 algorithm (Katoh *et al*. 2002; Katoh & Standley 2013) prior to phylogenetic analysis.

Genetic relationships based on ND2 and RAG-1 sequences from individuals collected in the ATL, GOM, and SUR were investigated using PAUP (version 4.0a152; Swofford 1998).

Parsimony trees were generated using a heuristic search with stepwise addition and 100 random addition sequence replicates to construct 50% majority rule consensus trees for ND2 and RAG-1, respectively. Parsimony analysis of 711 base pairs (bp) of the ND2 region from 65 individuals, including the closely related species *G. altavela* and two representatives from the Myliobatoidea (Southern Stingray, *Dasyatis americana* Hildebrand & Schroeder 1928; Bullnose Ray, *Myliobatis freminvillei* Lesueur 1824) and one outgroup taxon (Sandbar Shark, *Carcharhinus plumbeus* Nardo 1827), identified 282 parsimony-informative characters and recovered 4 equally parsimonious trees. RAG-1 parsimony analysis of 728 bp from 26 taxa including the Longsnout Butterfly Ray (*G. crebripunctata*), the Bat Ray (*M. californica* Gill 1865), the Spinetail Devil Ray (*Mobula japonica*, Müller & Henle 1841) and one outgroup (Spiny Dogfish, *Squalus acanthias* Linnaeus 1758) resulted in 28 parsimony-informative characters and 966 equally parsimonious trees. Support values for ND2 and RAG-1 consensus trees were calculated using bootstrap resampling with 1000 replicates of the data and a heuristic search with 100 random addition sequence replicates for each replicate (Felsenstein 1985).

To visualize genealogical relationships at the intraspecific level, haplotype networks were constructed for ND2 and RAG-1 sequence data. Sequences were collapsed into unique haplotypes as above and genetic relationships among species were inferred from sequence data using the Median Joining method of Bandelt *et al*. 1999, using the software package POPART (Leigh & Bryant 2015). Due to the limited amount of variation in RAG-1 sequences among individuals, sequences were collapsed into haplotypes using FaBox 1.41 (Villesen 2007), and a single exemplar of each unique sequence was retained for parsimony analysis. Geographic location of capture was included as a trait for each network.

Comparative material

Gymnura altavela (Linnaeus 1758). Western North Atlantic USA (16 specimens) – MCZ 160734.1, embryo female, 170 mm DW, North Carolina, 35°46' N, 75°30' W, 20 m depth, trawl, 9 Sep 2001; MCZ 160734.2, embryo female, 170 mm DW, North Carolina, 35°46' N, 75°30' W, 20 m depth, trawl, 9 Sep 2001; FLMNH 29994.5, female embryo, 363 mm DW, North Carolina, 33°47' N, 76°37' W, 47.5 m depth, 17 Mar 1961; FLMNH 29994.8, embryo male, 326 mm DW, North Carolina, 33°47' N, 76°37' W, 47.5 m depth, 17 Mar 1961; FLMNH 40709.2, female, 593 mm DW, North Carolina, 34°34' N, 76°28' W, 14.6 m depth, 13 May 1983; FLMNH 407019.3, female, 675 mm DW, North Carolina, 34°34' N, 76°28' W, 14.6 m depth, 13 May 1983; FLMNH 40709.1, juvenile male, 546 mm DW, North Carolina, 34°34' N, 76°28' W, 14.6 m depth, 13 May 1983; FLMNH 40709.6, juvenile male, 600 mm DW, North Carolina, 34°34' N, 76°28' W, 14.6 m depth, 13 May 1983; VIMS 34809, juvenile female, 730 mm DW fresh, Virginia, 37°9' N, 76°12' W, 10.1 m depth, trawl, 19 Sep 2014. Western South Atlantic Brazil (2 specimens) – MCZ S-581, female, 381 mm DW, Rio de Janeiro, 22° 53' S, 43° 17' W, Jan 1872 – Feb 1872; MNHN 2324, juvenile male, 305 mm DW, Rio de Janeiro, 23° 0' S, 43° 16' W (specimen labeled as a type specimen, but is not from the type locality for species). Eastern North Atlantic Mauritania (1 specimen) – MNHN 1989-1231, adult female, 718 mm DW, 20° 46' N, 16° 46' E, 6 m depth, Mar 1982. Eastern North Atlantic Senegal (2 specimens) – MNHN 1989-1241, embryo female, 380 mm DW, 14° 19' N, 17° 1' W, 9 m depth, Apr 1979; MNHN 1981-0114, embryo male, 390 mm DW, 14° 19' N, 17° 1' W, 9 m depth, Apr 1979. Eastern North Atlantic Guinea (1 specimen) – USNM 202761, adult male, 528 mm DW, 12° 10' S, 17° 4' W, 0 to 20 m depth, trawl, 11 Dec 1963. Eastern North Atlantic Côte d'Ivoire (1 specimen) – MNHN 1981-0115, juvenile male, 488 mm DW, 4° 19' N, 7° 22' W, 40 m depth, Mar 1979.

Gymnura australis (Ramsay & Ogilby 1886). Western South Pacific Australia (1 specimen) – USNM 39978, female, 650 mm DW, New South Wales.

Gymnura crebripunctata (Peters 1869). Eastern North Pacific Mexico (2 specimens) – USNM 28298, adult male, 287 mm DW, Sinaloa; FMNH 62351, juvenile male, 279 mm DW, Baja California, 23 Sep 1954.

Gymnura japonica (Temminck & Schlegel 1850). Western Pacific China (1 specimen) (1 specimen) – MNHN 6554, juvenile female, 268 mm DW, 25°0' N, 125°0' E. Western Pacific Korea (1 specimen) – FMNH 59307, juvenile male, 517 mm DW, Fusan.

Gymnura marmorata (Cooper 1864). Eastern North Pacific USA (6 specimens) – USNM 62384, female, 391 mm DW, California; USNM 62382, female, 439 mm DW, California; FMNH 33738, female, 327 mm DW, California, 1933; FMNH 42576, female, 297 mm DW, California, 32°42' N, 117°14' W, seine, 26 Jul 1945; FMNH 52254, female, 209 mm DW, California, 24 May 1911; USNM 8101, juvenile male, 361 mm DW, California.

Gymnura poecilura (Shaw 1804). Indian Sri Lanka (2 specimens) – FMNH 58888, juvenile male, 317 mm DW, Jan 1914; MCZ S-808, adult male, 371 mm DW, Ceylon, 7°38' N, 79°46' E, received Jan 1884. Indian Malaysia (1 specimen) – MCZ S-242, juvenile male, 287 mm DW, Penang, 5°26' N, 100°16' E, 1 Feb 1860 – 31 Aug 1860.

Gymnura tentaculata (Müller & Henle 1841). Indian India (2 specimens) – MNHN 2329, juvenile male, 245 mm DW, 1836; MNHN 2013-1220, juvenile female, 276 mm DW, Malabar, 11°0' N, 76°0' E.

Gymnura zonura (Bleeker 1852). Western Pacific China (1 specimen) – USNM 86007, juvenile female, 210 mm DW, Fukien. Western Pacific Indonesia (1 specimen) – MNHN 4997, juvenile male, 287 mm DW, Java, 6°7' S, 106°45' E. Indian Malaysia (1 specimen) – MCZ S-

245, female, 271 mm DW, Singapore, 1°20’ N, 103°50’ E. Pacific Taiwan (1 specimen) – MNHN 2013-0461, adult male, 542 mm DW, Tashi, 13 Mar 2012.

Material examined but not retained. *Gymnura altavela.* Western North Atlantic USA (17 specimens) KPGAVT8-1, juvenile male, 573 mm DW, Virginia, 37°9’ N, 76°2’ W, 7.6 m depth, trawl, 5 Sep 2013; KPGAVT25-1, juvenile female, 575 mm DW fresh, Virginia, 37°12’ N, 76°2’ W, 9.4 m depth, trawl, 1 Oct 2013; KPGAVT21-1, male, 506 mm DW fresh, Virginia, 37°11’ N, 76°8’ W, 7.9 m depth, trawl, 3 Jul 2013; KPGAVT1611-1, juvenile female, 427 mm DW fresh, Virginia, 36°58’ N, 76°1’ W, 14 m depth, trawl, 8 Jul 2016; KPGAB71, juvenile male, 549 mm DW fresh, North Carolina, 35°11’ N, 75°3’ W, 32 m depth, trawl, 11 Sep 2013; KPGAB74, juvenile male, 628 mm DW fresh, North Carolina, 35°4’ N, 75°9’ W, 69 m depth, trawl, 11 Sep 2013; KPGAB109, adult male, 1278 mm DW fresh, Virginia, 37°23’ N, 75°12’ W, 17 m depth, trawl, 14 Sep 2013; KP20152, adult female, 1843 mm DW fresh, North Carolina, 34°54’ N, 75°48’ W, 26 m depth, trawl, 18 Mar 2015; KPGA1376-1, juvenile male, 585 mm DW fresh, Virginia, 38°1’ N, 75°13’ W, 9.1 m depth, trawl, 26 Oct 2013; KPGA1388-1, juvenile female, 590 mm DW fresh, Virginia, 37°23’ N, 75°34’ W, 10.1 m depth, trawl, 28 Oct 2013; KPGA1396-1, juvenile female, 549 mm DW fresh, Virginia, 37°6’ N, 75°42’ W, 11 m depth, trawl, 29 Oct 2013; KPGA13105-1, adult female, 1905 mm DW fresh, Virginia, 36°56’ N, 75°44’ W, 14.9 m depth, trawl, 29 Oct 2013; KPGA13X-1, juvenile male, 628 mm DW fresh, Virginia, 37°28’ N, 75°14’ W, 29 m depth, trawl, 29 Oct 2013; KPGA13122-3, adult female, 1311 mm DW fresh, North Carolina, 35°37’ N, 75°23’ W, 17.1 m depth, trawl, 11 Nov 2013; KPGA56-1, juvenile female, 682 mm DW, Delaware, 38°40’ N, 74°58’ W, 18.4 m depth, trawl, 12 Oct 2012; KPGA85-1, juvenile female, 567 mm DW, Virginia, 37°41’ N, 75°29’ W, 13.4 m depth, trawl, 15 Oct 2012; KPGA85-4, juvenile male, 620 mm DW, data same as KPGA85-1.

Morphometric analysis

Canonical correlation analysis indicated significant variation in morphometric characters of both male and female among geographic regions. For males, the full suite of metrics significantly explained variability between regions ($F = 11.96$, $p = 0.001$), and collectively accounted for 22% of the variability in morphology. The first and second canonical axes (CA1, CA2) accounted for 80% and 20% of the explained variation, respectively, and were statistically significant (CA1 $F = 19.06$, $p = 0.001$; CA2 $F = 4.87$, $p = 0.002$). LNC had the greatest influence on the separation of regional groups on CA1 followed by LAPV, and SP loaded most heavily on CA2 (Fig. 3a). The strong negative loading of LNC corresponded to the centroid of GOM specimens, which had longer nasal curtains relative to ATL and SUR specimens, while the strong positive loading of SP reflects pelvic-fin spans that are broader in ATL specimens than GOM and SUR specimens (Table 2). Similarly, the positive loading of LAPV on CA1 and negative loading on CA2 aligns with the longest pelvic fins that are observed in SUR (Table 2). Overlap in the overall morphometric variation between ATL and GOM corresponds to greater morphometric similarity between these regions relative to SUR (Fig. 3b). To further explore regional differences between metrics, a second CCA was evaluated without LNC. The remaining metrics collectively accounted for 17% of morphological variation, which differed significantly between regions ($F = 8.95$, $p = 0.001$). Metric variation explained by CA1 and CA2 was 73% and 27%, respectively, and statistically significant (CA1 $F = 13.13$, $p = 0.001$; CA2 $F = 4.76$, $p = 0.003$). The removal of LNC from the analysis had minimal impacts on the separation of regional centroids, but increased overlap of metrics variation between regions (Fig. 3c–3d), and suggests that a combination of LNC, LAPV, and SP metrics best differentiates regional groups of male rays.

Regional differences in female morphometric characters were significant (F = 10.12, p = 0.001), and metrics collectively accounted for 18% of the total variation present. CA1 and CA2 were statistically significant (CA1 F = 15.83, p = 0.001; F = 4.40, p = 0.003), and accounted for 78% and 22% of the explained variation, respectively. LNC and SP strongly loaded positively on CA1 and CA2 along with the centroid for ATL specimens, while the centroid for GOM loaded positively on CA1 and negatively on CA2 (Fig. 4a). This pattern reflects larger lengths of these characters in ATL relative to GOM specimens (Table 2). The negative loading of the SUR centroid on CA1 corresponds to smaller LNC and SP in this region, and the generally low overlap of variation in metrics between SUR and the other two regions supports the morphological distinction of *G. micrura* from the northern congeners (Fig. 4a, Table 2). Removal of LNC from subsequent analyses corresponded to only a 2% loss in the total variation explained by the model (16%). Variation due to region remained statistically significant (F = 8.82, p = 0.001), and CA1 and CA2 were statistically significant (CA1 F = 12.55, p = 0.001; CA2 F = 5.10, p = 0.005), accounting for 71% and 29% of the total variation explained.

Molecular analysis

The final majority rule consensus trees for ND2 and RAG-1 sequences recovered three monophyletic lineages that correspond to ATL, GOM, and SUR specimens of *Gymnura micrura*. Western Atlantic *G. altavela* were recovered as genetically distinct from one sequence reported for a specimen from the type locality, and were therefore assigned *Gymnura* cf. *altavela* (Fig. 5–6). Bootstrap results (i.e., percentages based on 1000 trials) for the ND2 tree suggested high reliability in the topology, and grouped ATL, GOM, and SUR *Gymnura* sequences into three

distinct clades with 100% bootstrap support for each node (Fig. 5). Support for branch nodes in the RAG-1 tree were less robust; there was 66% support for the GOM clade (Fig. 6).

The ND2 haplotype network presented in Fig. 7 demonstrates that the magnitude of divergence between ATL, GOM and SUR specimens was significant (indicated by the large number of hash marks, or nucleotide differences, between regions), and greater than the differences within each region, corroborating the presence of unique taxa within the species complex. The ND2 network also suggests that SUR *G. micrura* is more closely related to the GOM species than to the ATL species (Fig. 7). The RAG-1 network clearly separated GOM and ATL *Gymnura* species, but failed to resolve the phylogenetic placement of SUR *G. micrura* based on the low number of parsimony informative characters available (Fig. 7).

Systematic account

***Gymnura micrura* (Bloch & Schneider 1801)**
Smooth Butterfly Ray

Figs. 8–9; Tables 1–2

Synonyms

Raja micrura Bloch & Schneider 1801: 360.

Trygon micrura Müller 1837: 40.

Pteroplatea maclura Müller & Henle 1841: 169.

Pteroplatea micrura Engelhardt 1913: 103.

Gymnura altavela Fowler 1945: 162.

Neotype: USNM 440357, adult male, 330 mm DW fresh, Suriname, 06°30’ N, 54°29’ W, 20-25 m depth, shrimp trawl, 5 Oct 2015. A holotype was not designated in the original description of *G. micrura*. Therefore, to preserve the current usage of *G. micrura* and to offer a point of comparison for this study, we designate a neotype (USNM 440357) for this species that was collected in the present study from the type locality (Suriname, South America)

Paratypes (16 specimens) - USNM 440356, female, 582 mm DW fresh, Suriname, 06°24’ N, 54°29’ W, 20-25 m depth, shrimp trawl, 3 Oct 2015; MCZ 171858, adult male, 333 mm DW fresh, 328 mm DW preserved, Suriname, 06°30’ N, 54°29’ W, 20-25 m depth, shrimp trawl, 5 Oct 2015; MCZ 171857, female, 442 mm DW fresh, 434 mm DW preserved, Suriname, 06°26’ N, 54°32’ W, 20-25 m depth, shrimp trawl, 3 Oct 2015; FLMNH 238555, adult male, 351 mm DW fresh, Suriname, 06°30’ N, 54°30’ W, 20-25 m depth, shrimp trawl, 3 Oct 2015; FLMNH 238688, female, 370 mm DW fresh, Suriname, 06°26’ N, 54°32’ W, 20-25 m depth, shrimp trawl, 3 Oct 2015; NZCS F 7099, female, 406 mm DW fresh, Suriname, 06°30’ N, 54°29’ W, 20-25 m depth, shrimp trawl, 5 Oct 2015; VIMS 35366, male, 348 mm DW fresh, Suriname, 06°30’ N, 54°29’ W, 20-25 m depth, shrimp trawl, 5 Oct 2015; VIMS 35367, adult male, 325 mm DW fresh, Suriname, 06°30’ N, 54°29’ W, 20-25 m depth, shrimp trawl, 5 Oct 2015; VIMS 35368, female, 419 mm DW fresh, Suriname, 06°30’ N, 54°29’ W, 20-25 m depth, shrimp trawl, 5 Oct 2015; VIMS 35369, adult male, 319 mm DW fresh, Suriname, 06°30’ N, 54°29’ W, 20-25 m depth, shrimp trawl, 5 Oct 2015; VIMS 35370, juvenile female, 395 mm DW fresh, Suriname, 06°26’ N, 54°32’ W, 20-25 m depth, shrimp trawl, 3 Oct 2015; VIMS 35371, adult male, 337 mm DW fresh, Suriname, 06°30’ N, 54°29’ W, 20-25 m depth, shrimp trawl, 5 Oct 2015; VIMS 35372, female, 498 mm DW fresh, Suriname, 06°30’ N, 54°29’ W, 20-25 m depth, shrimp trawl, 5 Oct 2015; VIMS 35373, female, 357 mm DW fresh, Suriname, 06°30’ N, 54°29’ W, 20-25 m depth, shrimp trawl, 5 Oct 2015; VIMS 35374, adult female, 561 mm DW fresh, Suriname,

06°26’ N, 54°32’ W, 20-25 m depth, shrimp trawl, 3 Oct 2015; VIMS 35375, female, 484 mm DW fresh, Suriname, 06°26’ N, 54°32’ W, 20-25 m depth, shrimp trawl, 3 Oct 2015.

Non-type specimens examined (69 specimens) – Caribbean Sea Mexico (1 specimen) - MCZ 37159, juvenile male, 220 mm DW, Carmen, 25°56’ N, 111°8’ W. Caribbean Sea Venezuela (4 specimens) - MCZ 51065, adult male, 303 mm DW, 10°29’ N, 62°21’ W, 14.6 m depth; MCZ 51051, juvenile male, 170 mm DW, 10°29’ N, 62°21’ W, 9.1 m depth; USNM 222908 (n = 2), all juveniles, female, 245 mm DW, male, 205 mm DW, 08°55’ N, 60°10’ W, 10.7 m depth, otter trawl, 27 Feb 1978. Western North Atlantic Suriname (19 specimens) – FLMNH 224447 (n = 2), juvenile male, 271 mm DW, adult male, 319 mm DW, 6°40’ N, 54°4’ W, 7 m depth, 10 Jul 1968; FMNH 89990, juvenile male, 244 mm DW; FMNH 89991, female, 214 mm DW, 18 m depth, 3 May 1957; MCZ 40414, juvenile male, 293 mm DW, 6°24’ N, 54°27’ W, 31.1 m depth, 15 Sep 1958; USNM 205354, female, 485 mm DW, 6°22’ N, 55°22’ W, 29 m depth, 30 Apr 1969; USNM 156716 (n = 4), all juveniles, female, 184 mm, female, 221 mm DW, male, 266 mm, female, 277 mm DW, 6°24’ N, 55°1’ W, 27 m depth; USNM 156714 (n = 9), female, 331 mm DW, female, 301 mm, juvenile male, 289 mm DW, juvenile female, 275 mm DW, juvenile female, 248 mm DW, juvenile male, 222 mm DW, juvenile male, 221 mm DW, juvenile male, 230 mm DW, juvenile female, 250 mm DW, 6°20’ N, 54°56’ W, 26 m depth, 30 May 1957. Western North Atlantic French Guiana (6 specimens) – USNM 222622, juvenile male, 317 mm DW, 4°25’ N, 50°55’ W, 37 to 40 m depth, twin flat trawls, 7 May 1975; USNM 222615, juvenile male, 233 mm DW, 4°42’ N, 51°28’ W, 0 to 37 m depth, twin flat trawls, 6 May 1975; USNM 222616, male, 242 mm DW, 4°25’ N, 50°55’ W, 37 to 40 m depth, twin flat trawls, 7 May 1975; FLMNH 41642, female, 385 mm DW, 4°58’ N, 51°58’ W, 32.9 m depth, 3 Jul 1972; FLMNH 35336, juvenile male, 231 mm DW, 5°5’ N, 51°58’ W, 11 Dec 1977; FLMNH 101740, adult male, 295 mm DW, 4°30’ N, 51°30’ W, 21 Feb 1968. Western North Atlantic

Brazil (7 specimens) – USNM 222597 (n = 5), all juveniles, female, 373 mm female, 319 mm DW, male, 287 mm DW, male, 277 mm DW, male, 182 mm DW, 0°48’ N, 47°45’ W, 46 to 48 m depth, 14 May 1975; MCZ 40417, juvenile male, 265 mm DW, 2°29’ N, 48°58’ W, 14 Nov 1957; MCZ 40402, female, 234 mm DW, 2°31’ N, 49°10’ W, 27.4 m depth, 14 Nov 1958. Western South Atlantic Brazil (2 specimens) – MNHN 7972, juvenile male, 350 mm DW, 12°58’ N, 38°28’ W; USNM 156822, juvenile male, 306 mm DW, 2°28’ N, 48°55’ W, 42 m depth, 15 Nov 1957. Eastern North Atlantic Senegal (8 specimens) – FLMNH 176854, female, 545 mm DW, Fatick, 14°00’ N, 14°00’ W, fish market, 8 Dec 2009; MNHN 1989-1216, male, 489 mm DW, Saloum, 15°00’ N, 18°00’ W, 14 m depth, May 1983; MNHN 1989-1225, adult female, 660 mm DW, Saloum, 15°00’ N, 18°00’ W, 10 m depth, May 1983; MNHN 1989-1220, juvenile male, 202 mm DW, 12°58’ N, 16°52’ W, 10 m depth, Feb 1980; MNHN 1989-1214, juvenile male, 215 mm DW, Saloum, 15°00’ N, 18°00’ W, 12 m depth, May 1983; MNHN 1989-1224, adult male, 400 mm DW, Saloum, 15°00’ N, 18°00’ W, 12 m depth, May 1983; MNHN 1989-1217, adult male, 525 mm DW, Saloum, 15°00’ N, 18°00’ W, 12 m depth, May 1983; MNHN 1989-1223, adult male, 446 mm DW, Saloum, 15°00’ N, 18°00’ W, 12 m depth, May 1983. Eastern North Atlantic Guinea-Bissau (1 specimen) – MNHN 1989-1232, adult female, 731 mm DW, Cacheu, 12°12’ N, 16°10’ W, 3 m depth, Aug 1983. Eastern North Atlantic Guinea (1 specimen) – MNHN 1985-0237, juvenile male, 279 mm DW. Eastern North Atlantic Sierra Leone (2 specimens) – FLMNH 29993, female, 507 mm DW, Upper Sierra Leone River, 8°34’ N, 13°5’ W, 3.7 to 5.5 m depth, 7 Feb 1968; USNM 279558, juvenile female, 286 mm DW, St. Anne Banana Islands, Feb 1986. Eastern North Atlantic Liberia (4 specimens) – USNM 193896, male 335 mm DW, Bushrod Island, 30 Oct 1952; USNM 193741, male, 379 mm DW, St. Paul River, 7 to 13 m depth, 6 Jan 1953; USNM 222623 (n = 2), all juveniles, female, 285 mm DW, female, 309 mm DW, 6°17’ N, 10°49’ W, 20 m depth, trawl, 8 Nov 1963. Eastern North Atlantic Côte d’Ivoire (4 specimens) – MNHN 1989-1227, male, 394 mm DW, 5°12’ N, 3°49’ W, 20 m depth,

Mar 1979; MNHN 1989-1226, female 475 mm DW, 5°12' N, 4°21' W, 15 m depth, Mar 1979; MNHN 1989-1229, female, 320 mm DW, 5°04' N, 3°48' W, 15 m depth, Mar 1979; MNHN 1989-1242, juvenile male, 210 mm DW, 5°04' N, 3°48' W, 15 m depth, Mar 1979. Eastern North Atlantic Ghana (1 specimen) – MNHN 222600, juvenile female, 179 mm DW, Tema, 19 Jan 1962. Eastern North Atlantic Togo (3 specimens) – MNHN 1989-1244, adult female 660 mm DW, 6°13' N, 1°37' E, 12 m depth, Jun 1983; MNHN 1989-1245, adult female, 692 mm DW, 6°13' N, 1°37' E, 12 m depth, Jun 1983; MNHN 1989-1247, adult female, 750 mm DW, 6°13' N, 1°37' E, 12 m depth, Jun 1983. Eastern North Atlantic Benin (2 specimens) – MNHN 1969-0211, juvenile male, 310 mm DW, 6°21' N, 2°54' E, 15 m depth, 19 Mar 1964; MNHN 1967-0737, juvenile male, 203 mm DW, 6°19' N, 2°24' E, 15 m depth, Jul 1964. Eastern North Atlantic Nigeria (2 specimens) – MNHN 1985-0217, juvenile female, 298 mm DW, 3°49' N, 6°13' E, 90 m deep, 26 May 1956; USNM 198011, female, 327 mm DW, Lagos. Eastern South Atlantic Gabon (1 specimen) – KPGMGabon, male, 375 mm DW fresh, 2°16' S, 9°30' E, 13.5 m depth, shrimp trawl, 10 Aug 2015. Eastern South Atlantic Angola (1 specimen) – FMNH 118133, juvenile male, 365 mm DW, 11°16' S, 13°42' E, 20 m depth, trawl, 6 Mar 2002.

Diagnosis. Dimensions as percentages of DW are given in Table 1 and Table 2. Diagnosis and description based on juvenile and adult male specimens.

Gymnura micrura is distinguished from other western Atlantic *Gymnura* by the combination of the following characters: a rhomboid disk, 1.5 to 1.9 times wider than long (1.8 to 1.9 times in females) and a short snout, pre-orbital snout length 8.8 – 14.2% of DW; moderate head length, one third of disk length; nasal curtain short (1.6% of DW), nasal curtain length 10.0 – 16.5% of pre-oral snout length; posterior pectoral length 75.2 – 87.3% of anterior pectoral length; pelvic span 37.9 – 51.8% 1st gill transverse distance; tail moderately short (36 – 77 mm) without a serrated spine, about one quarter of total length (23.0 – 29.4% TL) and 32.3% body

length; in life dorsal surface marbled and marginal white spots absent; ventral surface pale copper to golden yellow and occasionally marbled with creamy white near midline, gills, or pectoral tips; darker to dusky coloration near mid-pectoral margins of large specimens; dorsal tail surface with three to four well-defined light crossbars, and posteriormost dark crossbar sometimes extending across ventral surface of tail.

***Description*.** Disk rhomboidal in shape, 1.5 to 1.9 times wider than long. Anterior margin medially concave and weakly convex before apex, anterior pectoral length 57.2 – 69.2% of DW; apex acutely pointed; posterior margin straight to moderately convex and weakly rounded near insertion, posterior pectoral length 45.5 – 53.2 % of DW. Moderate head length, about one third of disk length. Eyes small and barely elevated, interorbital width (7.9 – 9.7% DW) less than interspiracular width (8.3 – 9.7% DW); spiracle tentacle absent. Pelvic fins triangular with angular free rear tip that extends beyond inner margin, anterior pelvic length 6.9 – 11.5% DW. Tail moderately short, 25.1% of total length, with low finfold, low ventral keel, and vestigial dorsal fin rarely present. Claspers short and slightly dorsally depressed, tapering distally. Inner margin of the clasper straight, lateral margin slightly convex medially; left clasper outer length 1.5 – 6.4% DW, left clasper inner length 4.7 – 13.5% DW.

Mouth width (7.9 – 9.1% DW) broader than internarial width (5.5 – 7.3% DW), preoral snout length 10.1 – 15.9% DW. Symphysal region of the lower jaw smooth and flat to weakly concave, becoming arched laterally and slightly concave near corners. Upper jaw medially concealed by nasal curtain. Nostril openings subovate and slanting anterolaterally, interior margin concealed by nasal curtain. Nasal curtain short (1.2 – 2.1% DW) and moderately narrow (6.6 – 8.2% DW), medially straight with moderately rounded posterolateral apices, prenarial snout length 8.0 – 12.8% DW. First gill slits posterior to mouth, with origins lateral of mouth corners and distance between successive gills slits decreasing; distance between 1st gill slits and snout

relatively short (17.7% DW), and transverse distance between 1st gill slits (16.0% DW) 1.5 times transverse distance between 5th gill slits (10.8% DW).

Coloration. In fresh specimens, dorsal surface taupe to rosy brown, densely covered with lighter, large and irregular spots and blotches, and interspersed with smaller and darker brown spots, giving marbled appearance. Ventral surface pale copper to golden yellow with darker to dusky coloration near mid-pectoral margins of large specimens, and occasionally marbled with creamy white near midline, gills, or pectoral tips. Dorsolateral margin of pelvic fins white. Dorsal surface of tail with three to four well-defined light crossbars; ventral surface same as ventral disk surface, with posteriormost dark crossbar extending across ventral surface in some specimens. In preserved specimens, dorsal surface is uniformly light to dark brown, with faint marbled blotches occasionally retained and smaller dispersed dark spots rarely retained; ventral surface retains marbled pattern that is generally faded to pale pinkish orange, yellow, or white.

Size. A small species of western Atlantic *Gymnura*, reaching a maximum disk width of 351 mm in males and 582 mm in females. Males between 170-289 mm DW are immature; maturity was observed in males wider than 290 mm. Females 184-395 mm DW are immature, and only two specimens were mature at 561 and 582 mm DW. Size at birth is unknown. The disk width of the smallest post-embryonic specimens was 184-221 mm.

Distribution. Northern coast of South America including at least Venezuela, Guyana, Suriname, and French Guiana; northwestern range boundary unknown. Currently includes Brazil, however genetic confirmation of the taxonomic identity of specimens occurring in the western South Atlantic is needed.

Etymology. The generic name is derived from a combination of the words naked and tail (Greek *gymnos* and *oura*), and the specific name *micrura* is a combination of the Greek *micro* and *oura*, in reference to the short tail.

***Remarks*.** Coloration may be highly variable both between and within juvenile and adult life stages (Fig. 14). Several morphometric differences occur between males and females, as demonstrated in Table 2.

***Gymnura* sp. nov. A**

Figures 10–11; Tables 1–2

Synonyms

Gymnura micrura Bloch & Schneider 1801

Gymnura sp. nov. A Parsons 2017

Holotype. USNM 440358, adult male, 406 mm DW, North Carolina, 36°13’ N, 75°45’ W, 8.8 m depth, trawl, 9 Nov 2013.

Paratype. USNM 440359, adult female, 638 mm DW, Georgia, 32°31’ N, 80°30’ W, 6.4 m depth, shrimp trawl, 25 Jun 2015.

Non-type material examined. Western North Atlantic USA (28 specimens) – MCZ 37059, adult female, 745 mm DW, North Carolina, 34°27’ N, 76°4’ W, 44 m depth; MCZ 37060,

adult female, 769 mm DW, North Carolina, 34°27’ N, 76°4’ W, 44 m depth; MCZ S-1344, juvenile male, 229 mm DW, North Carolina, 34°48’ N, 76°19' W; MCZ S-1345, juvenile male, 266 mm DW, North Carolina, 34°48’ N, 76°19' W; MCZ S-239 juvenile female, 222 mm DW, South Carolina, 32°45’ N, 79°52’ W, 1847-1853; MNHN A-7938, juvenile female, 210 mm DW, New York, 40°40’ N, 73°49’ W, 1823; USNM 42502, adult male, 399 mm DW, Virginia; FLMNH 29943, adult female, 728 mm DW, Florida, 28°42’ N, 80°42’ W, 20 Nov 1976; FLMNH 29981, adult female, 693 mm DW, Florida, 28°51’ N, 80°49’ W, 15 Jul 1976; FLMNH 233554, female, 496 mm DW, Florida, 25°42’ N, 80°14’ W, 30 Nov 1947; FLMNH 208562, juvenile male, 222 mm DW, Florida, 25°43’ N, 80°13’ W, shrimp trawl, 5 Apr 1958; FLMNH 29947, juvenile female, 305 mm DW, Florida, 28°42’ N, 80°42’ W, 2.8 m depth, 20 Apr 1978; FLMNH 47495, juvenile male, 304 mm DW, Florida, 28°24’ N, 80°34’ W, 19 Dec 1978; FLMNH 143163, juvenile male, 287 mm DW, Georgia, 30°56’ N, 81°26’ W, 14 Apr 1959; FLMNH 101784, juvenile male, 252 mm DW, Georgia, 31°58’ N, 80°34’ W, 8.2 m, 11 Dec 1960; FLMNH 29977, juvenile female, 361 mm DW, Georgia, 31°3’ N, 81°24’ W, 6 Jul 1959; FLMNH 184152, juvenile female, 254 mm DW, North Carolina, 22 May 1973; FMNH 18014, adult male, 402 mm DW, North Carolina; VIMS 35246, adult female, 655 mm DW fresh, Georgia, 31°49’ N, 80°57’ W, 6.4 m, shrimp trawl, 17 Jun 2015; VIMS 35254, female, 420 mm DW fresh, North Carolina, 36°13’ N, 75°45’ W, 8.8 m depth, trawl, 9 Nov 2013; VIMS 35255, juvenile male, 239 mm DW fresh, Virginia, 36°59’ N, 76°19’ W, 19.2 m depth, trawl, 17 Sep 2014; VIMS 35256, juvenile female, 276 mm DW fresh, Virginia, 37°38’ N, 76°2’ W, 21 m depth, trawl, 15 Sep 2014; VIMS 35258, adult female, 690 mm DW fresh, Georgia, 31°49’ N, 80°57’ W, 6.4 m depth, shrimp trawl, 17 Jun 2015; VIMS 35264, adult female, 789 mm DW fresh, Georgia, 31°49’ N, 80°57’ W, 6.4 m depth, shrimp trawl, 17 Jun 2015; VIMS 35265, adult female, 810 mm DW fresh, Georgia, 31°49’ N, 80°57’ W, 6.4 m depth, shrimp trawl, 17 Jun 2015; VIMS 35269, adult female, 690 mm DW fresh, Georgia, 31°49’ N, 80°57’ W, 7 m depth, shrimp trawl, 16 Jun 2015;

VIMS 34829, adult female, 635 mm DW fresh, Georgia, 31°49’ N, 80°57’ W, 6.4 m depth, shrimp trawl, 17 Jun 2015; KPGM15121, female, 407 mm DW fresh, North Carolina, 35°29’ N, 75°25’ W, 15.3 m depth, trawl, 4 May 2015.

Material examined but not retained. Western North Atlantic USA (33 specimens) – KPGMVT101-1, juvenile male, 300 mm DW fresh, Virginia, 37°0’ N, 76°20’ W, 7.6 m depth, trawl, 7 Aug 2013; KPGMVT35-1, adult male, 410 mm DW fresh, Virginia, 37°26’ N, 76°12’ W, 7.9 m depth, trawl, 2 Oct 2013; KPGMVT24-1, adult male, 435 mm DW fresh, Virginia, 37°20’ N, 76°2’ W, 7.9 m depth, trawl, Sep 3 2013; KPGMVT2-1, adult female, 610 mm DW fresh, Virginia, 37°6’ N, 76°10’ W, 11 m depth, trawl, 2 Oct 2014; KPGMVT14-1, adult male, 453 mm DW fresh, Virginia, 36°58’ N, 76°1’ W, 14.6 m depth, trawl, 1 Oct 2013; KPGMVT7-1, male, 380 mm DW fresh, Virginia, 37°0’ N, 76°20’ W, 7.6 m depth, trawl, 16 Jul 2013; KPGMVT32-1, adult male, 420 mm DW fresh, Virginia, 37°23’ N, 76°10’ W, 15.2 m depth, trawl, 5 Aug 2013; KPGM13107-1, adult female, 797 mm DW fresh, Virginia, 36°43’ N, 75°49’ W, 14.3 m depth, trawl, 30 Oct 2013; KPGMTT02, adult female, 506 mm DW fresh, Georgia, 31°49’ N, 80°57’ W, 6.4 m depth, shrimp trawl, 17 Jul 2015; KPJXM502-1, juvenile female, 221 mm DW fresh, Florida, 30°27’ N, 81°26’ W, 1.2 m depth, haul seine, 8 Aug 2013; KPJXM408-1, female, 450 mm DW fresh, Florida, 30°31’ N, 81°30’ W, 2 m depth, trawl, 5 Sep 2013; KPJXM409-1, adult female, 624 mm DW fresh, Florida, 30°34’ N, 81°29’ W, 5.7 m depth, trawl, 12 Nov 2013; KPJXM201-1, adult male, 367 mm DW fresh, Florida, 30°42’ N, 81°26’ W, 1.7 m depth, haul seine, 23 Aug 2013; KPJXM201-2, adult female, 835 mm DW fresh, Florida, 30°42’ N, 81°26’ W, 1.7 m depth, haul seine, 23 Aug 2013; KPJXM201-3, adult female, 681 mm DW fresh, Florida, 30°42’ N, 81°26’ W, 1.7 m depth, haul seine, 23 Aug 2013; KPJXM201-4, adult female, 725 mm DW fresh, Florida, 30°42’ N, 81°26’ W, 1.7 m depth, haul seine, 23 Aug 2013; KPJXM304-1, juvenile female, 220 mm DW fresh, Florida, 30°32’ N, 81°29’ W, 2.5 m depth,

haul seine, 29 Oct 2013; KPJXM304-2, juvenile male, 255 mm DW fresh, Florida, 30°32’ N, 81°29’ W, 2.5 m depth, haul seine, 29 Oct 2013; KPJXM304-3, juvenile male, 246 mm DW fresh, Florida, 30°32’ N, 81°29’ W, 2.5 m depth, haul seine, 29 Oct 2013; KPJXM304-4, juvenile female, 246 mm DW fresh Florida, 30°32’ N, 81°29’ W, 2.5 m depth, haul seine, 29 Oct 2013; KPJXM304-5, juvenile female, 305 mm DW fresh, Florida, 30°42’ N, 81°31’ W, 2.3 m depth, haul seine, 21 Aug 2013; KPJXM102-3, male, 294 mm DW fresh, Florida, 30°42’ N, 81°31’ W, 2.3 m depth, haul seine, 21 Aug 2013; KPGM1378-1, adult male, 487 mm DW fresh, Virginia, 37°46’ N, 75°27’ W, 10.7 m depth, trawl, 27 Oct 2013; KPGM1389-1, female, 504 mm DW fresh, Virginia, 37°24’ N, 75°38’ W, 7.6 m depth, trawl, 28 Oct 2013; KPGM1391-1, juvenile male, 395 mm DW fresh, Virginia, 37°14’ N, 75°43’ W, 8.2 m depth, trawl, 28 Oct 2013; KPGM1397-1, adult male, 420 mm DW fresh, Virginia, 37°8’ N, 75°46’ W, 7.9 m depth, trawl, 28 Oct 2013; KPGM1390-1, juvenile female, 478 mm DW fresh, Virginia, 37°19’ N, 75°39’ W, 10.1 m depth, trawl, 28 Oct 2013; KPGM13X-1, adult male, 485 mm DW fresh, Virginia, 37°28’ N, 75°14’ W, 29 m depth, trawl, 29 Oct 2013; KPGM13109-1, adult female, 725 mm DW fresh, Virginia, 36°38’ N, 75°49’ W, 15.2 m depth, trawl, 30 Oct 2013; KPGM13118-1, adult female, 740 mm DW fresh, North Carolina, 36°29’ N, 75°43’ W, 14.3 m depth, trawl, 30 Oct 2013; KPGM13107-2, adult female, 870 mm DW fresh, Virginia, 36°43’ N, 75°49’ W, 14.3 m depth, trawl, 30 Oct 2013; KPUNF13-2, juvenile female, 265 mm DW fresh, Florida, 30°43’ N, 81°31’ W, 6.2 m depth, bottom longline, 30 May 2013; KPGM1395-1, juvenile female, 544 mm DW fresh, Virginia, 37°16’ N, 75°40’ W, trawl, 28 Oct 2013. .

***Diagnosis*.** Dimensions as percentages of DW are given in Table 1 and Table 2. Diagnosis and description based on juvenile and adult male specimens.

Gymnura sp. nov. A is distinguished from other western Atlantic *Gymnura* by the combination of the following characters: a rhomboid disk, 1.5 to 2.0 times wider than long (1.7 to

1.9 times in females) and a short snout, pre-orbital snout length 9.7 – 14.8% of DW; moderately long head length, greater than one third of disk length; nasal curtain moderately short (1.8% of DW), nasal curtain length 11.1 – 18.6% pre-oral snout length; posterior pectoral-fin length 74.1 – 92.1% of anterior pectoral-fin length; pelvic-fin span 38.1 – 56.2% 1st gill transverse distance; tail short (32 – 80 mm) without a serrated spine, less than one quarter of total length (17.6 – 24.9% TL) and 25.1% of body length; dorsal surface with light and fine vermiculate pattern, speckled with numerous small and irregular creamy white spots, and disk margins lined with creamy white spots; ventral surface uniformly white, occasionally fading to pale yellow near posterior pectoral margins, with darker to dusky coloration near mid-pectoral margins of large specimens; dorsal tail surface with two to five light crossbars that are mottled in large specimens.

***Description*.** Disk rhomboidal in shape, 1.5 to 2.0 times wider than long. Anterior margin moderately concave medially and straight to slightly convex before apex, anterior pectoral length 55.5 – 66.9% of DW; apex acutely pointed; posterior margin straight to weakly convex and weakly rounded near insertion, posterior pectoral-fin length 47.7 – 53.4% of DW. Moderate head length, greater than one third of disk length. Eyes small and barely elevated, interorbital width (7.8 – 11.3% DW) less than interspiracular width (8.7 – 10.5% DW); spiracle tentacle absent. Pelvic fins subtriangular with slightly rounded free rear tip, posterior margin straight to weakly angular, with lateral margin slightly shorter than inner margin, anterior pelvic length 6.6 – 9.2% DW. Tail short, 20.8% of total length, with moderately low dorsal finfold and ventral keel. Claspers short and conical, tapering distally. Clasper inner margin straight, lateral margin slightly convex medially; left clasper outer length 2.1 – 7.4% DW, left clasper inner length 3.0 – 13.1% DW.

Mouth width (8.0 – 10.2% DW) broader than internarial width (5.7 – 7.0% DW), preoral snout length 10.7 – 15.5% DW. Lower jaw symphysal region moderately concave, broadly

arched laterally. Upper jaw medially concealed by nasal curtain. Nostril openings subovate and slanting anterolaterally, interior margin concealed by nasal curtain. Nasal curtain moderately short (1.3 – 2.5% DW) and moderately narrow (6.8 – 9.1% DW), medially straight with moderately rounded posterolateral apices, prenarial snout length 8.7 – 13.6% DW. First gill slits posterior to mouth, with origins lateral of mouth corners and distance between successive gills slits decreasing; distance between 1st gill slits and snout relatively short (17.8% DW), and transverse distance between 1st gill slits (15.7% DW) 1.4 times transverse distance between 5th gill slits (11.3% DW).

Coloration. In fresh specimens, dorsal surface light to dark brown, grey, or olive green with lighter, fine vermiculate pattern, speckled with numerous small and irregular creamy white spots and larger tan to brown ephemeral spots of variable size, occasionally with large dark spots dispersed symmetrically in pairs of one to three on posterior half of disk; conspicuous creamy white spots line entire disk margin; ventral surface white fading to pale yellow near posterior pectoral margins, with darker to dusky coloration near mid-pectoral margins of large specimens; dorsolateral margin of pelvic fins white, extending along posterior margin in small specimens; dorsal tail surface with two to five light crossbars that are mottled in large specimens, ventral surface same as ventral disk surface. In preserved specimens, dorsal surface is uniformly light to dark tan or brown with yellowish, creamy white dispersed speckles and marginal spots (may be faded in very small and very large specimens), often without evidence of faint brown, grey, or black spots; ventral surface uniformly pinkish white or light grey; tail banding pattern retained.

Size. A large species of the western Atlantic Gymnuridae reaching a maximum disk width of 870 mm in males and 1040 mm in females (NEFSC, unpublished data); maximum sizes of 487 and 838 mm DW were observed during the present study. Males between 222–395 mm

DW were immature, and maturity was observed in males wider than 367 mm. Females between 210–478 mm DW were immature, and maturity was observed in females wider than 506 mm DW. Maximum observed fecundity is six, and size at birth is estimated between 205-239 mm DW (Parsons 2017).

Distribution. Western North Atlantic, New Jersey to the southeast Florida coast in the US.

Etymology. TBD.

Remarks. Coloration is highly variable both between and within juvenile and adult life stages (Fig. 14). Ephemeral spots disappear with removal of dermal mucous during capture and post-mortem. Marginal white spots are retained throughout life, but may darken in large specimens. Several morphometric differences occur between males and females (Table 2), and become more dissimilar during ontogeny. Notably, the anterolateral angulation of the posterior margin of the pelvic fins, and elongation of the preorbital snout length of males occurs during and post-maturation relative to females that have straight posterior pelvic fin margins and retain a broad and relatively short snout (Fig. 10–11).

***Gymnura* sp. nov. B**

Figures 12–13; Tables 1–2

Synonyms

Gymnura micrura Bloch & Schneider 1801

Gymnura sp. nov. B Parsons 2017

Holotype. Gulf of Mexico, USA – USNM 440360, adult male, 331 mm DW fresh, Florida, 30°1' N, 84°22' W, 30 April 2016.

Paratype (1). USNM 440361, adult female, 694 mm DW fresh, Alabama, 6 to 12 m depth, trawl, Jun to Jul 2013.

Non-type material examined. Gulf of Mexico USA (91 specimens) – FLMNH 36834 (n = 2), juvenile female, 546 mm DW, female, 424 mm DW, Florida, 26°2' N, 81°46' W, 3.7 m depth, 1 Mar 1979; FLMNH 79937, adult male, 385 mm DW, Florida, 29°51' N, 85°23' W, 1.8-3.7 m depth, 7 Jun 1989; FLMNH 51168 (n = 2), all juveniles, female, 415 mm DW, male, 252 mm DW, Florida, 29°54' N, 84°30' W, 6 Sep 1952; FLMNH 180332, adult male, 368 mm DW, Florida, 26°42' N, 82°10' W, 12 Oct 1963; FLMNH 159627, adult male, 351 mm DW, Florida, 25°1' N, 80°22' W, 23 Feb 1965; FLMNH 74623, juvenile male, 214 mm DW, Florida, 25°57' N, 81°43' W, Aug 1974; FLMNH 50363, juvenile female, 208 mm DW, Florida, 29°53' N, 84°21' W, 20 May 1951; FLMNH 65132, juvenile female, 246 mm DW, Florida, 26°10' N, 81°48' W, 8 Aug 1966; FLMNH 826, adult male, 295 mm DW, Florida, 29°5' N, 83°3' W, 27 Mar 1954; FLMNH 2112, juvenile male, 243 mm DW, Florida, 29°7' N, 83°3' W, 7 Jun 1950; FLMNH 56231 (n = 3), all juveniles, male, 208 mm DW, female, 238 mm DW, male, 283 mm DW, Florida, 29°51' N, 84°37' W, 28 Apr 1960; FLMNH 74554, embryo male, 88 mm DW, Florida, 1 Aug 1974; FLMNH 56254, embryo male, 137 mm DW, Florida, 29°51' N, 84°37' W, 3 Oct 1959; FLMNH 73634 (n = 2), all embryos, male, 141 mm DW, female, 157 mm DW, Florida, 29°36' N, 84°57' W, 26 Oct 1974; FLMNH 51265 (n = 2), all embryos; male, 106 mm DW, female, 105 mm DW, Florida, 29°54' N, 84°30' W, 6 Sep 1952; FLMNH 224493, juvenile

female, 318 mm DW, Florida, 25°32’ N, 81°14’ W, 4 Nov 1965; FLMNH 119176, adult male, 377 mm DW, Florida, 24°45’ N, 82°43’ W, 18.3 m depth, 4 May 1989; FLMNH 81692, female 530 mm DW, Florida, 24°43’ N, 82°10’ W, 19 m depth, 21 May 1989; FMNH 10636, adult male, 378 mm DW, Louisiana, 3 May 1957; FMNH 10745 (n = 2), all embryos, female, 105 mm DW, female, 105 mm DW, Louisiana; FMNH 31889, juvenile male, 208 mm DW, Mississippi, 20 May 1933; FMNH 11188, juvenile male, 159 mm DW, Texas, 1924; FMNH 37811 (n = 2), female, 403 mm DW, adult male, 320 mm DW, Texas, 1936; FMNH 11187, juvenile female, 290 mm DW, Texas, 1924; MCZ S-95, female, 375 mm DW, Florida, 26°31’ N, 82°11’ W, received 1857-59; MCZ 51060 (n = 4), all embryos; male, 170 mm DW, male, 185 mm DW, female, 188 mm DW, female, 180 mm DW, Louisiana, 28°47’ N, 90°24’ W, 18.3 to 21.9 m depth; USNM 127299, juvenile female, 233 mm DW, Florida; USNM 127334, female, 257 mm DW, Louisiana; USNM 143221, female, 326 mm DW, Mississippi; USNM 94545 (n = 2), all adults, male, 324 mm DW, male, 332 mm DW, Texas; USNM 127073, juvenile female, 285 mm DW, Texas, 0 to 18 m depth, 26 to 27 Feb 1917; VIMS 35235, adult male, 346 mm DW fresh, Florida, 30°1’ N, 84°22’ W, 30 April 2016; VIMS 35236, adult male, 331 mm DW fresh, Florida, 30°1’ N, 84°22’ W, 30 April 2016; VIMS 34826, adult male, 341 mm DW fresh, Florida, 30°1’ N, 84°22’ W, 30 April 2016; VIMS 34827, adult male, 397 mm DW fresh, Florida, 30°1’ N, 84°22’ W, 30 April 2016; VIMS 34828, adult male, 377 mm DW fresh, Florida, 30°1’ N, 84°22’ W, 30 April 2016; KPGMFSUBB16-1, adult male, 430 mm DW fresh, Florida, 29°56’ N, 83°20’ W, 1.8 m depth, gillnet, 16 Aug 2016; KPGMFSUBB16-2, adult male, 406 mm DW fresh, Florida, 29°23’ N, 83°16’ W, 1.6 m depth, 17 Aug 2016; VIMS 34811, adult male, 360 mm DW fresh, Alabama, 6 to 12 m depth, trawl, Jun to Jul 2013; VIMS 34812, adult male, 383 mm DW fresh, data same as VIMS 34811; VIMS 34813, adult male, 355 mm DW fresh, data same as VIMS 34811; VIMS 34814, juvenile male, 303 mm DW fresh, data same as VIMS 34811; VIMS 35244, adult female, 612 mm DW fresh, data same as VIMS 34811; VIMS 35249, adult male, 294 mm DW fresh, data

same as VIMS 34811; VIMS 35248, juvenile male, 364 mm DW, fresh data same as VIMS 34811; VIMS 35273, adult male, 354 mm DW fresh, data same as VIMS 34811; VIMS 35247, adult male, 422 mm DW fresh, data same as VIMS 34811; VIMS 35270, adult female, 838 mm DW fresh, data same as VIMS 34811; VIMS 35260, adult female, 684 mm DW fresh, data same as VIMS 34811; VIMS 35262, adult female, 675 mm DW fresh, data same as VIMS 34811; VIMS 35257, adult female, 705 mm DW fresh, data same as VIMS 34811; VIMS 35272, adult female, 654 mm DW fresh, data same as VIMS 34811; VIMS 35266, adult female, 709 mm DW fresh, data same as VIMS 34811; VIMS 35268, adult female, 746 mm DW fresh, data same as VIMS 34811; VIMS 35237, adult female, 563 mm DW fresh, data same as VIMS 34811; VIMS 35245, adult female, 520 mm DW fresh, data same as VIMS 34811; VIMS 35263, adult female, 754 mm DW fresh, data same as VIMS 34811; VIMS 35240, adult female, 518 mm DW fresh, data same as VIMS 34811; VIMS 35242, adult female, 566 mm DW fresh, data same as VIMS 34811; VIMS 35243, adult female, 694 mm DW fresh, data same as VIMS 34811; VIMS 35252, adult female, 642 mm DW fresh, data same as VIMS 34811; VIMS 35271, adult female, 725 mm DW fresh, data same as VIMS 34811; VIMS 35250, adult female, 567 mm DW fresh, data same as VIMS 34811; VIMS 35259, adult female, 704 mm DW fresh, data same as VIMS 34811; VIMS 35267, adult female, 825 mm DW fresh, data same as VIMS 34811; VIMS 35238, adult male, 379 mm DW fresh, data same as VIMS 34811; VIMS 35239, adult male, 412 mm DW fresh, data same as VIMS 34811; VIMS 35241, adult female, 495 mm DW fresh, data same as VIMS 34811; VIMS 35253, juvenile male, 242 mm DW fresh, data same as VIMS 34811; VIMS 35261, adult female, 694 mm DW fresh; VIMS 34816, adult male, 366 mm DW fresh, data same as VIMS 34811; VIMS 34818, adult male, 346 mm DW fresh, data same as VIMS 34811; VIMS 34820, female, 451 mm DW fresh, data same as VIMS 34811; VIMS 34821, juvenile male, 276 mm DW fresh, data same as VIMS 34811; VIMS 34822, adult male, 378 mm DW fresh, data same as VIMS 34811; VIMS 34823, adult male, 393 mm DW fresh, data same as VIMS 34811;

VIMS 34824, adult male, 377 mm DW fresh, data same as VIMS 34811; VIMS 34825, adult male, 407 mm DW fresh, data same as VIMS 34811.

Specimens examined but not retained. Gulf of Mexico USA (35 specimens) – KPGMFSU814-1, juvenile female, 354 mm DW fresh, Florida, 29°53' N, 84°30' W, seine, 14 Aug 2014; KPGMFSU814-2, juvenile female, 321 mm DW fresh, Florida, 29°53' N, 84°30' W, seine, 14 Aug 2014; KPTXGM13-1, juvenile female, 341 mm DW fresh, Texas, 26°9' N, 97°17' W, 0.7 m depth, gillnet, 1 Oct 2013; KPTXGM13-2, juvenile female, 387 mm DW fresh, Texas, 26°5' N, 97°12' W, 2.1 m depth, trawl, 7 Oct 2013; KPTXGM13-5, adult female, 720 mm DW fresh, Texas, 26°10' N, 97°15' W, 2 m depth, trawl, 7 Oct 2013; KPTXGM13-4, adult female, 707 mm DW fresh, Texas, hook and line, Oct 2013; KPTXGM13-3, juvenile female, 260 mm DW fresh, Texas, 26°7' N, 97°16' W, 0.4 m depth, gillnet, 2 Oct 2013; KPGMAL0713-10, juvenile female, 291 mm DW fresh, Alabama, 6 to 12 m depth, trawl, Jun to Jul 2013; KPGMAL0713-11, juvenile female, 475 mm DW fresh, data same as KPGMAL0713-10; KPGMAL0713-12, adult male, 370 mm DW fresh, data same as KPGMAL0713-10; KPGMAL0713-13, adult male, 459 mm DW fresh, data same as KPGMAL0713-10; KPGMAL0713-14, juvenile male, 250 mm DW fresh, data same as KPGMAL0713-10; KPGMAL0713-15, juvenile male, 270 mm DW fresh, data same as KPGMAL0713-10; KPGMAL0713-16, adult male, 352 mm DW fresh, data same as KPGMAL0713-10; KPGMAL0713-17, adult male, 353 mm DW fresh, data same as KPGMAL0713-10; KPGMAL0713-18, adult male, 404 mm DW fresh, data same as KPGMAL0713-10; KPGMAL0713-19, adult male, 369 mm DW fresh, data same as KPGMAL0713-10; KPGMAL0713-24, adult male, 435 mm DW fresh, data same as KPGMAL0713-10; KPGMAL0713-25, adult female, 532 mm DW fresh, data same as KPGMAL0713-10; KPGMAL0713-26, adult female, 556 mm DW fresh, data same as KPGMAL0713-10;

KPGMAL0713-39, adult male, 425 mm DW fresh, data same as KPGMAL0713-10; KPGMAL0713-43, adult male, 380 mm DW fresh, data same as KPGMAL0713-10; KPGMAL0713-40, female, 484 mm DW fresh, data same as KPGMAL0713-10; KPGMAL0713-41, adult male, 376 mm DW fresh, data same as KPGMAL0713-10; KPGMAL0713-38, adult female, 568 mm DW fresh, data same as KPGMAL0713-10; KPGMAL0713-60, adult female, 655 mm DW fresh, data same as KPGMAL0713-10; KPGMAL0713-46, adult female, 612 mm DW fresh, data same as KPGMAL0713-10; KPGMAL0713-54, adult male, 415 mm DW fresh, data same as KPGMAL0713-10; KPGMAL0713-53, adult male, 343 mm DW fresh, data same as KPGMAL0713-10; KPGMAL0713-48, adult male, 414 mm DW fresh, data same as KPGMAL0713-10; KPGMAL0713-37, adult female, 544 mm DW fresh, data same as KPGMAL0713-10; KPGMAL0713-31, adult female, 620 mm DW fresh, data same as KPGMAL0713-10; KPGMFSU0612-1, male, 377 mm DW fresh, Florida, 29°53' N, 84°30' W, gillnet, 1 Jun 2012; KPGMMML-1, juvenile female, 260 mm DW fresh, Florida, 26°32' N, 82°7' W, 2.8 m depth, gillnet, 22 Jul 2015; KPGMMMK16, female, 585 mm DW fresh, Florida, 29°44' N, 84°56' W, gillnet, 2012; KPGMMMK17, female, 445 mm DW fresh, data same as KPGMMK16.

Diagnosis. Dimensions as percentages of DW are given in Table 1 and Table 2. Diagnosis and description based on juvenile and adult male specimens.

Gymnura sp. nov. B is distinguished from other western Atlantic *Gymnura* by the combination of the following characters: a rhomboid disk, 1.5 to 2.0 times wider than long (1.7 to 2.0 times in females) and a moderate snout, pre-orbital snout length 8.7 – 15.9% of DW; head length moderately short, less than one third of disk length; nasal curtain medium to short (2.1% of DW), nasal curtain length 12.0 – 18.8% pre-oral snout length; posterior pectoral length 72.5 – 87.6% of anterior pectoral length; pelvic span 29.3 – 51.9% of 1st gill transverse distance; tail

short (11 – 90 mm) without a serrated spine, less than one quarter of total length (11.0 – 26.3% TL) and 26.2% body length, caudal spine absent; dorsal surface with mosaic of dark brown minute spots becoming fine vermiculate pattern in adults, and dark brown to black spots on posterior half of pectoral fins, dispersed symmetrically in one to three pairs, and marginal white spots absent; ventral surface pinkish white fading to yellowish gold from mid-pectoral to pectoral tip, or marbled yellowish gold throughout, with darker and dusky mid-anterior pectoral margin; dorsal tail surface with two to four light crossbars that become mottled in large specimens.

***Description*.** Disk rhomboidal in shape, 1.5 to 2.0 times wider than long. Anterior margin concave medially, and moderately convex before apex, anterior pectoral length 59.5 – 69.5% of DW; apex acutely pointed; posterior pectoral margin straight to weakly convex and rounded near insertion, posterior pectoral length 46.5 – 53.5 % of DW. Head length short, less than one third of disk length. Eyes small and barely elevated, interorbital width (7.6 – 11.1% DW) less than interspiracular width (8.8 – 11.1% DW); spiracle tentacle absent. Pelvic fins subtriangular with angular free rear tip, posterior margin straight to weakly angular, with lateral margin slightly shorter than inner margin, anterior pelvic length 5.6 – 9.6% DW. Tail short (11-90 mm) without serrated spine, 21.7% of total length and 26.2% body length, with low finfold and ventral keel. Claspers short and slightly conical, straight inner and lateral margins, tapering distally; left clasper outer length 1.7 – 7.6% DW, left clasper inner length 5.3 – 12.8% DW.

Mouth width (8.4 – 10.5% DW) broader than internarial width (6.0 – 8.4% DW), preoral snout length relatively long, 10.0 – 16.7% DW. Symphysal region of lower jaw slightly concave, broadly arched laterally. Upper jaw medially concealed by nasal curtain. Nostril openings subovate and slanting slightly anterolaterally, interior margin concealed by nasal curtain. Nasal curtain medium to short (1.5 – 2.5% DW) and relatively broad (6.4 – 10.0% DW), slightly concave posterior margin with rounded posterolateral apices, prenarial snout length 8.0 – 14.2%

DW. First gill slits posterior to mouth, with origins lateral to mouth corners and distance between successive gills slits decreasing; distance between 1st gill slits and snout relatively long (19.1% DW), and transverse distance between 1st gill slits (16.4% DW) 1.4 times transverse distance between 5th gill slits (12.1% DW).

Coloration. In fresh specimens, dorsal surface taupe to pinkish brown or greyish green, with mosaic of dark brown minute spots becoming fine vermiculate pattern in adults, and dark brown to black spots on posterior half of pectoral fins, dispersed symmetrically in pairs (typically between one and three pairs, but sometimes absent) and most prevalent near tail base, peppering of black spots of variable size in small specimens, and faint brown to grey spots occasionally dispersed throughout in large specimens; ventral surface pinkish white fading to yellowish gold from mid-pectoral to pectoral-fin tip, or marbled yellowish gold throughout, with darker and dusky mid-anterior pectoral margin; dorsolateral margin of pelvic fins white, extending along posterior margin in small specimens; dorsal tail surface with two to four light crossbars that become mottled in large specimens; ventral tail surface same as ventral disk surface. In preserved specimens, dorsal surface is uniformly light to dark tan or brown, typically with evidence of symmetrical faint brown to black spots but may be absent; ventral surface pinkish white, often retaining pale and darker yellowish orange marbled pattern; tail banding pattern retained.

Size. A medium-sized species of western Atlantic Gymnuridae, with observed maximum disk widths of 459 mm in males and 856 mm in females. Males between 159 – 364 mm DW were immature, and maturity was observed in males wider than 294 mm. Females between 208 – 387 mm DW were immature, and maturity was observed in females wider than 348 mm DW. Maximum observed fecundity is 12, and size at birth is estimated between 120 – 242 mm DW (Parsons 2017).

***Distribution*.** Northern Gulf of Mexico, Florida Keys to southern Texas border in the USA. Extent of southern range boundary unknown.

***Etymology*.** TBD.

***Remarks*.** Coloration is variable both between and within juvenile and adult life stages (Fig. 12–14). Spot pairs on posterior dorsal surface and faint dispersed spots are lost with removal of dermal mucous during capture and post-mortem. Several morphometric differences occur between males and females, as demonstrated in Table 2, and these differences become greater throughout ontogeny. Notably, the anterolateral angulation of the posterior margin of the pelvic fins, and elongation of the preorbital snout length of males occurs during and post-maturation relative to females that have straight posterior pelvic fin margins and retain a broad and relatively short snout (Fig. 12–13).

Key to species of western Atlantic Gymnuridae:

1 Tail with one or more serrated spines; posterior margin of spiracle with distinct tentacle ……..... *Gymnura altavela*

- Tail without serrated spines; no tentacle on posterior margin of spiracle 2

2 Dorsal disk marbled with irregular blotches; margin lacks white spots …….. *Gymnura micrura*

- Dorsal disk with white speckles and marginal white spots ..... *Gymnura* sp. nov. A

- Dorsal disk lacks white speckles and marginal white spot ..... *Gymnura* sp. nov. B

Discussion

Morphological variation of taxa is manifested through ontogenetic changes, differences due to sexual dimorphism and individual variability, and taxonomic characters (Grande 2004; Hilton & Bemis 2012). The present study identifies each category as contributing to the variability observed among specimens of western Atlantic *G. micrura*. Variation in overall body shape during ontogeny diverges by sex as gymnurids grow, and intraspecific inconsistencies in taxonomic characters (e.g., disk coloration, tail banding patterns, presence or absence of dorsal fin), have contributed to substantial taxonomic confusion within the family. *Gymnura micrura* was one of the first described species of Gymnuridae. In the original description, Bloch and Schneider (1801) provided few diagnostic details for the species from Suriname, except for a characterization of the tail as short, slender, and black- and white-barred; a holotype was not documented for reference or comparison to congeners. Consequently, this nominal species has been reported from several locations throughout the Atlantic and Indo-West Pacific, further obfuscating the validity of *G. micrura*. There has recently been significant progress in the

taxonomic resolution of the genus and several species based on morphological and molecular characters. Jacobsen & Bennett (2009) provided morphological and molecular evidence that all species belong to a single genus (*Gymnura*), thus reducing *Aetoplatea* (Valenciennes in Müller & Henle 1841) to a junior synonym. Definitive identification of the eastern Pacific congeners *G. crebripunctata* and *G. marmorata*, and delineation of their respective geographical distributions, was provided by Smith *et al*. (2009). In the western Pacific, differentiation of *G. bimaculata* from *G. japonica* based on the presence of dorsal spots was first contradicted by Isouchi (1977), followed by Shen *et al*. (2012) using mitochondrial DNA sequences, confirming the junior synonymy of the former with *G. japonica*. In the Indo-Pacific, *G. poecilura* (Shaw) was re-described by Muktha *et al*. (2016), and ND2 and cytochrome C oxidase 1 (COI) data revealed that reports of *G. micrura* and *G. japonica* in the Indian Ocean were erroneous. An on-going family-level revision of Gymnuridae will help to address many of the remaining taxonomic issues of this family (Yokota, pers. comm., 2017).

In a study of the life history of *G. micrura* that began in 2012 (Parsons, 2017), it became clear that there was significant variation in reproductive biology, growth patterns, as well as morphology of *G. micrur*a from the northern Gulf of Mexico and the Atlantic coast of North America. In the absence of a type specimen, however, and adequate consideration for variation in the morphology of *G. micrura*, vague and often inaccurate information for the species has persisted from early descriptions to contemporary identification keys, biodiversity inventories, and population status assessments. Without species-specific information, effective management strategies for the conservation of populations and the biodiversity of ecosystems are challenging.

The three species of the proposed western Atlantic *G. micrura* complex are readily distinguished from *G. altavela* by the absence of spiracular tentacles and caudal spines. *Gymnura* sp. nov. A is easily differentiated from *G. micrura* and *Gymnura* sp. nov. B by the presence of white spots along the disk margin. Disk coloration differences between *Gymnura* sp. nov. B and

G. micrura may be less discernible in post-mortem specimens; fresh specimens of the latter generally have a marbled dorsal surface and a nearly uniform golden yellow ventral surface, with well-defined light crossbars on the tail, while fresh *Gymnura* sp. nov. B often present a small number of dark paired spots on the posterior dorsal surface and have less intense and discontinuous coloration on the ventral disk surface, and have tail crossbars that become mottled and indiscernible with age.

Multivariate analyses suggested that a combination of morphometrics can be used to classify specimens by their region of origin, but also revealed overlap in the variability of size-corrected measurements between regions, particularly among the newly described species. Variation in the majority of characters evaluated in the present study follow a trend in which size-corrected measurements were generally smallest in *G. micrura* and largest in *Gymnura* sp. nov. B among males. Female *G. micrura* also had the smallest character measurements relative to the two new species, and metrics from female *Gymnura* sp. nov. A were often larger than those recorded for *Gymnura* sp. nov. B. Despite attempts to include a representative range of sizes for both sexes by using both fresh and preserved material, data from early life stages and very large specimens was limited, and additional data from these size classes is needed to refine the range of %DW morphometrics presented here.

Nasal curtain length contributed most to the discrimination of species, however the magnitude of change in the size of this character during ontogeny is relatively small (i.e., < 20 mm) (Fig. 15). Nasal curtain length has previously been identified as the most significant character differentiating the eastern Pacific species *G. crebripunctata* and *G. marmorata* (Smith *et al*. 2009), and the present study supports the importance of this character in the identification of Gymnuridae. Although tail morphology and color pattern are often used to differentiate *Gymnura*, these characters were highly variable and inconsistent both within and between Atlantic specimens examined (Fig. 16), and are not reliable as diagnostic characters for the complex. The

otherwise conserved morphology of *Gymnura* requires complementary evaluation of genetic character divergence to accurately describe these cryptic species.

Mitochondrial and nuclear DNA analyses corroborated the phenotypic and geographic discontinuity within the western Atlantic species complex. Supplementary analyses based on COI and cyt-*b* sequences from *Gymnura* sp. nov. A and *Gymnura* sp. nov. B agreed with ND2 and RAG-1 results (data not presented here), further supporting the division of Atlantic and Gulf of Mexico individuals into unique taxa that are genetically and morphologically dissimilar to *G. micrura* from the northern coast of South America. Genotype and haplotype data analysis also recovered US *G. altavela* from the western Atlantic as genetically distinct from an eastern Atlantic *G. altavela* (GenBank NADH2 sequence JQ518833), concurring with previous reports that the western Atlantic population is probably an undescribed species (Naylor *et al*. 2012; Weigmann 2016). Although direct morphometric analysis of eastern Atlantic *Gymnura* was outside the scope of the present study, morphometrics of *G. micrura* from this region clearly differ from the three species evaluated (see Table 2), and the observed geographical variation in metrics and traits warrant closer investigation. Interestingly, some eastern Atlantic morphotypes shared the white marginal disk spots diagnostic of the western Atlantic *Gymnura* sp. nov. A, although spots were generally smaller, fewer in number, and dispersed along the margin at greater intervals in the former (Fig. 17).

Preserved material from the southern distribution of *G. micrura* (e.g., Brazil) was examined during this study. However, genetic material was unavailable for direct comparison, and thus morphometrics for Brazil specimens were excluded from the canonical correlation analysis. The coastal dynamics of northern Brazil are strongly influenced by the freshwater and sediment discharge from the Amazon River, and this area represents the largest source of riverine sediment input into the world ocean (Degens *et al*. 1991). Interactions between the turbid freshwater plume from the Amazon and the northwestern flowing North Brazil Current divert

most of the sediment north, where it is deposited in mudbanks along the northern coast of South America (Kuehl *et al*. 1986; Peterson & Stramma 1991; Allison *et al*. 2000), and provides habitat preferred by gymnurids (e.g. Last *et al*. 2016). The Amazon shelf area has been considered a biogeographical barrier to coastal marine species, and is hypothesized to play a role in the genetic divergence between Brazilian and Caribbean populations of many invertebrates and fishes, including spiny lobster (*Panulirus argus* Latreille), ocean surgeonfish (*Acanthurus bahianu* Castelnau), and the endemic Brazilian large-eyed stingray (*Hypanus marianae* Gomes, Rosa & Gadig) (Sarver *et al*. 1998; Rocha *et al*. 2002; Rocha 2003; Rosa & Furtado 2004; Yokota & Lessa 2006). Given the potential but unknown influence of this barrier on the distribution of *G. micrura* from Suriname, and in the absence of genetic material, the southern range boundary for the species cannot be inferred from the present study and needs confirmation. Future efforts to resolve uncertainty in the taxonomic status of southwest and eastern Atlantic *Gymnura* will benefit from complimentary morphological and molecular analyses, and are crucial for delineating the range of distribution of *G. micrura*, and determining the true biodiversity of this group.

Accurate taxonomy provides the foundation for all biological studies of species, and is essential for: (1) addressing knowledge gaps of chondrichthyan species (and others) that are poorly known, and 2) improving the conservation management of all species to maintain the biodiversity and overall health of ecosystems worldwide. The vulnerability of coastal batoid populations to threats, including direct and indirect fishing pressure and habitat degradation, is dependent on specifies-specific life history strategies, ecological considerations, and the current size of populations—information that remains largely unknown for nearly one quarter of all batoids that are considered data deficient (Dulvy *et al*. 2014), including *G. micrura, Gymnura* sp. nov. A, and *Gymnura* sp. nov. B. Interspecific differences reported in the life histories of these species, including maximum size, size at reproductive maturity, and fecundity (Yokota & Lessa

2006, 2007; Yokota *et al*. 2012; Parsons 2017) require species-specific assessments and management considerations. Although the US populations are presently categorized as species of Least Concern based on sparse population data and presumed low post-release mortality from indirect fisheries (Grubbs & Ha 2006), other Atlantic gymnurids with similar life histories are threatened (Vooren *et al*. 2007; Walls *et al*. 2015). Off South Africa, mortality rates near 50% were reported for *G. natalensis* due to shrimp trawl bycatch between 1989 and 1992 (Fennessy 1994). Decades of intense coastal fishing pressure in the southwest Atlantic off the coast of Brazil have also contributed to the depletion of *G. altavela*, such that the species is now Critically Endangered in this region (Vooren *et al*. 2007). In US waters, *Gymnura* sp. nov. A may be more vulnerable to indirect fishing mortality than *Gymnura* sp. nov. B due to its larger size, potential older age at sexual maturity, and lower fecundity (Parsons 2017), since recruitment to fishing gear before successfully reproducing is likely greater. Reductions in the bottom trawl bycatch of large, reproductively mature Butterfly Rays have been demonstrated through the use of bycatch reduction devices. In the shrimp trawl fisheries off Suriname, for example, the bycatch of large *G. micrura* was reduced by 32% in trawls with turtle exclusion devices (TEDs) relative to trawls without TEDs (Willems 2013), and offers a promising solution for decreasing the risk of post-release mortality and stress impacts on gymnurids and other batoid species vulnerable to mobile fishing gears. Without empirical data on the physiological impacts of capture and release on the Gymnuridae, conservative management measures are encouraged and re-assessment of all species should be prioritized to address data deficiencies, and to evaluate potential threats to discrete populations with geographic distributions that are much smaller than previously thought. Careful consideration of taxonomic and biological information for each Atlantic species is vital to facilitate the effective management and conservation of populations in US waters.

Acknowledgements

We are grateful to the captains and crew of the following survey programs for their contribution of specimens, data, and knowledge of US Butterfly Ray populations: Chesapeake Bay Multispecies Monitoring and Assessment Program (ChesMMAP), the Northeast Area Monitoring and Assessment Program (NEAMAP), the Virginia Shark Monitoring and Assessment Program (VASMAP), and the Juvenile Fish and Blue Crab Survey (Virginia Institute of Marine Science, Department of Fisheries Science); Northeast Fisheries Science Center Multispecies Bottom Trawl Survey; Southeast Area Monitoring & Assessment Program (SEAMAP) Bottom Trawl Survey and the Sea Turtle Trawl Survey (South Carolina Department of Natural Resources, Marine Resources Research Institute); Cooperative Atlantic States Shark Pupping & Nursery (COASTSPAN) Bottom Longline Survey (University of North Florida, Shark Biology Program); Fisheries Independent Monitoring Program Bottom Trawl Survey (Florida Fish and Wildlife Research Institute, Jacksonville, FL); Gulf of Mexico Shark Pupping & Nursery (GULFSPAN) Bottom Longline & Gillnet Survey (Florida State University Coastal and Marine Laboratory, St. Teresa, FL); Dauphin Island Marine Laboratory (University of South Alabama, Dauphin Island, AL); Coastal Fisheries Division Bottom Trawl Survey (Texas Parks & Wildlife Department, Corpus Christi, TX). We especially thank J. Ruhle, D. Ward, J. Gartland , C. Bonzek, J. Galbraith, P. Chase, G. Mears, C. Ward, K. Spanik, J. Gregg, D. Gregg, D. Gauthier, M. Chattin, T. Tuckey, W. Lowery, E. Loose, J. Olney Jr., V. Hogge, D. Grubbs, M. Kolmann, A. Kroetz, G. Graeber, T. DiGirolamo, R. Brodie, J. Gelsleichter, M. McCallister, L. Parker, J. Dingle, J. Schwenter, M. Arendt, F. Hernandez, S. Hall, T. Willems, K. Wan, J. White, K. Aguiar Gabaldo, T. Nalovic, J. Rudloe, C. Rudlow, and B. Fisher for the collection or preparation of specimens and data. We thank the following museum collections for access to preserved specimens, and the staff for their curatorial assistance: National Museum of Natural History, Smithsonian Institution, Washington, D.C. (J. Williams, K. Murphy, S. Raredon, S. Smith); the Harvard University

Museum of Comparative Zoology, Cambridge, MA (K. Hartel, A. Williston); the Field Museum, Chicago, IL (S. Mochel, K. Swagel, C. McMahon); the Florida Museum of Natural History, Gainesville, FL (R. Robins, D. Boyd, Z. Randall); Muséum National d'Histoire Naturelle, Paris, France (Z. Gabsi, N. Schnell); Nunnally Ichthyology Collection, Gloucester Point, VA (S. Huber). K. Parsons is particularly indebted to D. Grubbs, M. Kolmann, and L. Parenti for their insight and contributions to the inception of this study, and C. Sweetman, W. Vogelbein, and C. Cotton for their thoughtful comments on earlier versions of this manuscript. We also thank M. Seitz for performing molecular analyses during the initial phase of this project. This study was partially funded by the Ernst Mayr Travel Grant in Animal Systematics awarded to K. Parsons by the Museum of Comparative Zoology at Harvard University, and by a National Science Foundation Women in Science and Engineering (NSF WISE) grant awarded to J. McDowell. Research and travel was also supported by awards from the VIMS Graduate Student Association to K. Parsons. Funding for VIMS Survey Programs was provided by: NOAA Chesapeake Bay Office; the Virginia Environmental Endowment; the U.S. Fish and Wildlife Service; and the Virginia Marine Resources Commission (ChesMMAP); the Atlantic States Marine Fisheries Commission; the Mid Atlantic Fisheries Management Council; the Commercial Fisheries Research Foundation; and the NOAA Northeast Fisheries Science Center (NEAMAP); NOAA Fisheries (Silver Spring, MD) (VASMAP); the U.S. Fish and Wildlife Service and the Virginia Marine Resources Commission (Juvenile Fish and Blue Crab Survey). This is contribution number xxxx of the Virginia Institute of Marine Science, College of William and Mary.

Literature Cited

Alcock, A. (1892) Some observations on the embryonic history of *Pteroplataea micrura*. *Annals and Magazine of Natural History Series* 6, 10, 1–8, pl. 4.

Allison, M.A., Lee, M.T., Ogston, A.S. & Aller, R.C. (2000) Origin of Amazon mudbanks along the northeastern coast of South America. *Marine Geology*, 163, 241–256.

Amoroso, E.C. (1960) Viviparity in fishes. *In*: Jones, I.C. (Ed.) *Hormones in Fish*. Symposia of the Zoological Society No. 1. London, pp. 153–181.

Bandelt, H.J., Forster, P. & Röhl, A. (1999) Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution*, 16(1), 37–48.

Bigelow, H.B. & Schroeder, W.C. (1953) *Fishes of the Western North Atlantic. Sawfishes, Guitarfishes, Skates, Rays, and Chimaeroids, No. 1, Part 2*. Yale University Press. Memoirs Sears Foundation for Marine Research, Yale Univ., New Haven, CT.

Compagno, L.J.V., Ebert, D.A. & Smale, M.J. (1989) *Guide to the Sharks and Rays of Southern Africa*. New Holland Publishers , London.

Compagno, L.J.V. & Last, P.R. (1999) Gymnunridae. *In*: Carpenter K.E. & Niem, V.H. (Eds.), *FAO species identification guide for fishery purposes. The living marine resources of the Western Centra Pacific. Volume 3. Batoid fishes, chimeras and bony fishes part 1 (Elopidae to Linophrynidae)*. FAO, Rome, pp. 1397–2068.

Daiber, F.C. & Booth, R.A. (1960) Notes on the biology of the butterfly rays, *Gymnura altavela* and *Gymnura micrura*. *Copeia* 1960, 137–139.

Degens, E.T., Kempe, S. & Richey, J.E. (1991) Summary: biogeochemistry of major world rivers. *In*: Degens, E.T., Kempe, S. & Richey, J.E. (Eds.), *Biogeochemistry of major world rivers*. Wiley, New York, pp. 323–347.

Dulvy, N.K., Baum, J.K., Clarke, S., Compagno, L. J.V., Cortés, E., Domingo, A., Fordham, S., Fowler, S., Francis, M.P., Gibson, C., Martínez, J., Musick, J.A., Soldo, A., Stevens, J.D. & Valenti, S. (2008) You can swim but you can't hide: the global status and conservation of oceanic pelagic sharks and rays. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 18, 459–482. doi:10.1002/aqc.975

Dulvy, N.K., Fowler, S.L., Musick, J.A., Cavanagh, R.D., Kyne, P.M., Harrison, L.R., Carlson, J.K., Davidson, L.N., Fordham, S.V., Francis, M.P., Pollock, C.M., Simpfendorfer, C.A., Burgess, G.H., Carpenter, K.E., Compagno, L.J., Ebert, D.A., Gibson, C., Heupel, M.R., Livingstone, S.R., Sanciangco, J.C., Stevens, J.D., Valenti, S. & White, W.T. (2014) Extinction risk and conservation of the world's sharks and rays. *Elife*, Cambridge 3:e00590. doi:10.7554/eLife.00590

Eschmeyer, W.N., Fong, J.D. (2015) Species by family/subfamily in the Catalog of Fishes California Academy of Sciences (15 Feb 2015).

Felsenstein, J. (1985) Confidence limits on phylogenies: An approach using the bootstrap. *Evolution*, 39, 783–791.

Fennessy, S.T. (1994) Incidental capture of elasmobranchs by commercial prawn trawlers on the Tugela Bank, Natal, South Africa. *South African Journal of Marine Science*, 14, 287–296.

Grande, L. (2004) Categorizing various classes of morphological variation, and the importance of this to vertebrate paleontology. *In*: Arratia, G. & Tintori, A. (Eds.), *Mesozoic Fishes 3* –

Systematics, Paleoenvironments and Biodiversity. Verlag Dr. Friedrich, München, Germany, pp. 126–126.

Grubbs, R.D. & Ha, D.S. (2006) *Gymnura micrura*. The IUCN Red List of Threatened Species. Version 2014.2. www.iucnredlist.org. Downloaded on 23 September 2014.

Hilton, E.J. & Bemis, W.E. (2012) External morphology of shortnose sturgeon *Acipenser brevirostrum* (Acipenseriformes: Acipenseridae) from the Connecticut River, with notes on variation as a natural phenomenon. *In*: Kynard, B., Bronzi, P. & Rosenthal, H. (Eds.), *Life History and Behavior Connecticut River Shortnose and Other Sturgeons*. World Sturgeon Conservation Society, special publication 4, pp. 243–275.

Isouchi, T. (1977) Butterfly ray *Gymnura bimaculata*, a junior synonym of *G. japonica*. *Japanese Journal of Ichthyology*, 23(4), 242–244.

Jacobsen, I.P. & Bennett, M.B. (2009) A taxonomic review of the australian butterfly ray *Gymnura australis*, (Ramsay & Ogilby, 1886) and other members of the family Gymnuridae (Order Rajiformes) from Indo-West Pacific. *Zootaxa*, 2228, 1–28.

Katoh, Misawa, Kuma, Miyata (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research*, 30, 3059–3066.

Katoh, Standley (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution*, 30, 772–780.

Koressaar, T. & Remm, M. (2007) Enhancements and modifications of primer design program Primer3. *Bioinformatics*, 23, 1289–1291.

Kuehl, S.A., DeMaster, D.J. & Nittrouer, C.A. (1986) Nature of sediment accumulation on the Amazon continental shelf. *Continental Shelf Research*, 6, 209–225.

Last, P.R., Naylor, G., Serét, B., White, W., Stehmann, M., de Carvalho, M. (2016) Rays of the World. CSIRO Publishing, Collingwood, Australia.

Last, P.R., White, W.T. & Séret, B. (2016) Taxonomic status of maskrays of the *Neotrygon juhlii* species complex (Myliobatoidei: Dasyatidae) with the description of three new species from the Indo-West Pacific. *Zootaxa* 4083(4), 533–561.

Last, P.R., Naylor, G.J.P. & Manjaji-Matsumoto, B.M. (2016) A revised classification of the family Dayatidae (Chondrichthyes: Myliobatiformes) based on new morphological and molecular insights. *Zootaxa*, 4139(3), 345–368. http://doi.org/10.11646/zootaxa.4139.3.2.

Leigh, J.W. & Bryant, D. (2015) POPART: full-feature software for haplotype network construction. *Methods in Ecology and Evolution*, 6, 1110–1116.

McEachran, J.D. & de Carvalho, M.R. (2002) Batoid Fishes. *In*: Carpenter, K.E. (Ed.), *The living marine resources of the western Central Atlantic. Vol 1 Introduction, molluscs, crustaceans, hagfishes, sharks, batoid fishes and chimaeras. FAO Species Identification Guide for Fisheries Purposes and American Society of Ichthyologists and Herpetologists Special Publication No. 5*. FAO, Rome, pp. 508–589.

McEachran, J.D., Séret, B. (1990) Gymnuridae. *In*: Quero, J.C., Hureau, J.C., Karrer, C., Post, A. & Saldanha, L. (Eds.) *Check-list of the fishes of the eastern tropical Atlantic (CLOFETA), Vol 1*. JNICT, Lisbon; SEI, Paris; and UNESCO, Paris, pp. 64–66.

Muktha, M., Akhilesh, K.V., Sandhya, S., Jasmin, F., Jishnudev, M.A., Kizhakudan, S.J. (2016) Re-description of the longtail butterfly ray, *Gymnura poecilura* (Shaw, 1804) (Gymnuridae: Myliobatiformes) from Bay of Bengal with a neotype designation. *Marine Biodiversity*. doi 10.1007/s12526-016-0552-8

Murdy, E.O., Birdsong, R.S. & Musick, J.A. (1997) *Fishes of Chesapeake Bay*. Smithsonian Institution Press, Washington, D.C.

Murdy, E.O. & Musick, J.A. (2013) *Fishes of Chesapeake Bay*. Johns Hopkins University Press, Baltimore, MD.

Naylor, G.J.P., Ryburn, J.A., Ferigo, O. & Lopez, A. (2005) Phylogenetic relationships among the major lineages of modern elasmobranchs. *In*: Hamlett, W.C. (Ed.), *Reproductive biology andphylogeny of Chondrichthyes: sharks, batoids and chimaeras*. Science Publishers, Enfield, NH, pp. 1–25.

Naylor, G.J.P., Caira, J.N., Jensen, K., Rosana, K., White, W. & Last, P. (2012) A DNA sequence based approach to the identification of shark and ray species and its implications for global elasmobranch diversity and parasitology. *Bulletin of the American Museum of Natural History*, 367, 263 pp.

Parsons, K.T. (2017) *Age, growth and reproduction of western North Atlantic Butterfly Rays (Myliobatiformes: Gymnuridae), with the description of two new species*. Doctoral dissertation, College of William & Mary, School of Marine Science, Virginia Institute of Marine Science, Williamsburg, VA, 168 pp.

Peterson, R.G. & Stramma, L. (1991) Upper-level circulation in South Atlantic Ocean. *Progress in Oceanography*, 26, 1–73.

Ranzi, S. (1934) Le basi fisio-morfologiche dello sviluppo embrionale dei Selaci. Parti II e III. *Pubblication Stazione Zoologica di Napoli*, 13, 331–437.

Robins, C.R. & Ray, G.C. (1986) *A field guide to Atlantic coast fishes of North America*. Houghton Mifflin Company, Boston, 354 pp.

Rocha, L.A., Bass, A.L., Robertson, D.R. & Bowen, B.W. (2002) Adult habitat preferences, larval dispersal, and the comparative phylogeography of three Atlantic surgeonfishes (Teleostei: Acanthuridae). *Molecular Ecology*, 11, 243–252.

Rocha, L.A. (2003) Patterns of distribution and processes of speciation in Brazilian reef fishes. *Journal of Biogeography*, 30, 1161–1171.

Rosa, O.S.A. & FURTADO, M. (2004) *Dasyatis marianae*. *In*: IUCN 2012. IUCN Red List of Threatened Species. Version 2012.2. www.iucnredlist.org

Sarver, S.K., Silberman, J.D. & Walsh, P.J. (1998) Mitochondrial DNA sequence evidence supporting the recognition of two subspecies of the Florida spiny lobster *Panulirus argus*. *Journal of Crustacean Biology*, 18, 177–186.

Shen, A., Chunyan, M., Yong, N., Zhaoli, X. & Lingbo. M. (2012) The Taxonomic Status of *Gymnura bimaculata* and *G. japonica*: Evidence from Mitochondrial DNA Sequences. *Journal of Life Sciences*, 6, 9–13.

Smith, W.D., Bizzarro, J.J., Richards, V.P., Nielsen, J., Marquez-Farias, F. & Shivji, M.S. (2009) Morphometric convergence and molecular divergence: the taxonomic status and evolutionary history of *Gymnura crebripunctata* and *Gymnura marmorata* in the eastern Pacific Ocean. *Journal of Fish Biology*, 75, 761–783.

Swofford, D.L. (1998). PAUP: phylogenetic analysis using parsimony, version 4.0a152. Computer program distributed by Sinnaur Associates Inc., Sunderland, MA.

ter Braak, C.J.F. (1986) Canonical correspondence analysis : a new eigenvector technique for multivariate direct gradient analysis. *Ecology*, 67, 1167–1179.

Untergrasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B.C., Remm, M. & Rozen, S.G. (2012) Primer3 - New capabilities and interfaces. *Nucleic Acids Research*. 40, e115.

Villesen, P. (2007) FaBox: an online toolbox for FASTA sequences. *Molecular Ecology Notes*, 7, 965–968.

Vooren, C.M., Piercy, A.N., Snelson Jr., F.F., Grubbs, R.D., Notarbartolo di Sciara, G. & Serena, S. (2007) *Gymnura altavela*. The IUCN Red List of Threatened Species 2007: e.T63153A12624290. http://dx.doi.org/10.2305/IUCN.UK.2007.RLTS.T63153A12624290.en. Downloaded on 22 April 2017.

Walls, R., Vacchi, M., Notarbartolo di Sciara, G., Serena, F. & Dulvy, N. (2015) *Gymnura altavela*. The IUCN Red List of Threatened Species 2015: e.T63153A48931613. Downloaded on 22 April 2017.

Weigmann, S. (2016) Annotated checklist of the living sharks, batoids, and chimeras (Chondrichthyes) of the world, with a focus on biogeographical diversity. *Journal of Fish Biology*, 88(3), 837–1037. doi: 10.1111/jfb.12874

White, W.T., Last, P.R., Naylor, G.J.P. & Harris, M. (2010) Resurrection and redescription of the Borneo Broadfin Shark *Lamiopsis tephrodes* (Fowler, 1905) (Carcharhiniformes: Carcharhinidae). *In*: Last, P.R., White, W.T. & Pogonoski, J.J. (Eds.), *Description of New Sharks and Rays from Borneo*. CSIRO Marine and Atmospheric Research Paper 032, pp. 45–49.

Willems, T. (2013) By-catch of rays in the trawl fishery for Atlantic seabob shrimp *Xiphopenaeus kroyeri* in Suriname: how effective are TEDs and BRDs? *ILVO MEDEDELING* nr 139.

White, W.T. & Last, P.R. (2012) A review of the taxonomy of chondrichthyan fishes: a modern perspective. *Journal of Fish Biology*, 80, 901–917.

Wood-Mason, J. & Alcock, A. (1891) On the uterine villiform papillae of *Pteroplatea micrura* and their relation to their embryo. *Proceedings of the Royal Society of London*, 49, 359–367.

Yokota, L. & Lessa, R.P. (2006) A nursery area for sharks and rays in Northeastern Brazil. *Environmental Biology of Fishes*, 75, 349–360.

Yokota, L. & Lessa, R.P. (2007) Reproductive biology of three ray species: *Gymnura micrura* (Bloch & Schneider, 1801), *Dasyatis guttata* (Bloch & Schneider, 1801) and *Dasyatis mariana* (Gomes, Rosa & Gadig, 2000) caught by artisanal fisheries in Northeastern Brazil. *Cahiers de Biologie Marine*, 48, 249–247.

Yokota, L., Goitein, R., Gianeti, M.D. & Lessa, R.T.P. (2012) Reproductive biology of the smooth butterfly ray *Gymnura micrura*. *Journal of Fish Biology*, 81, 1315–1326.

Tables

TABLE 1. Morphometric values in mm and percentage of disk width (%DW) for adult *Gymnura micrura* from Suriname, *Gymnura* sp. nov. A from the U.S. western North Atlantic, and *Gymnura* sp. nov. B from the U.S. Gulf of Mexico. Abbreviations for morphometrics provided in Figure 2.

	Gymnura micrura				*Gymnura* sp. nov. A				*Gymnura* sp. nov. B			
	male neotype		female paratype		male holotype		female paratype		male holotype		female paratype	
	USNM 440357		USNM 440356		USNM 440358		USNM 440359		USNM 440360		USNM 440361	
	mm	%DW	mm	%DW	Mm	%DW	mm	%DW	mm	%DW	mm	%DW
DW	330		582		406		638		331		694	
LT	261	79.1	420	72.2	304	74.9	480	75.2	damaged		525	75.6
LAP	210	63.6	351	60.3	257	63.3	388	60.8	216	65.3	413	59.5
LPP	168	50.9	295	50.7	210	51.7	336	52.7	161	48.6	370	53.3
LB	210	63.6	323	55.5	246	60.6	396	62.1	207	62.5	407	58.6
LD	201	60.9	319	54.8	241	59.4	375	58.8	202	61.0	395	56.9
LH	68.4	20.7	94.4	16.2	79.2	19.5	101	15.8	72.7	22.0	116	16.6
LPOBS	42.8	13.0	52.3	9.0	48.3	11.9	60.8	9.5	45	13.6	66.2	9.5
WIO	27.4	8.3	46.6	8.0	33.1	8.2	59.9	9.4	32.1	9.7	60.9	8.8
WIS	28.6	8.7	48.1	8.3	39.2	9.7	61.5	9.6	32.5	9.8	60.6	8.7
LSV	175	53.0	273	46.9	209	51.5	343	53.8	178	53.8	349	50.3
LSG1	63.4	19.2	84.6	14.5	71.7	17.7	94.4	14.8	66	19.9	102	14.8
DG5	32.9	10.0	63.0	10.8	46	11.3	73.1	11.5	39.8	12.0	78.4	11.3
DG1	50.1	15.2	93.5	16.1	64.5	15.9	108	17.0	53.3	16.1	109	15.7
LAPV	28.7	8.7	40.2	6.9	32.6	8.0	55.9	8.8	25	7.6	53.3	7.7
SP	22.1	6.7	40.2	6.9	29.7	7.3	59.7	9.4	25	7.6	57.8	8.3
LPN	38.8	11.8	44.3	7.6	41.5	10.2	52.2	8.2	40.7	12.3	58.7	8.5
LPOLS	46.4	14.1	55.9	9.6	53	13.1	63.5	10.0	48.7	14.7	70.6	10.2
LNC	4.8	1.5	7.7	1.3	8.8	2.2	10.9	1.7	6.7	2.0	10.8	1.6
WIN	20.5	6.2	32.5	5.6	26.1	6.4	41.2	6.5	22.7	6.9	43.8	6.3
WNC	24.1	7.3	37.9	6.5	32.1	7.9	48.4	7.6	27.9	8.4	52.6	7.6
WM	27.7	8.4	46.2	7.9	36.2	8.9	60.8	9.5	31.9	9.6	64.9	9.4
ILCL	16.9	5.1			25.3	6.2			22.1	6.7		
OLCL	32.4	9.8			46.1	11.4			40.5	12.2		

TABLE 2. Morphometrics for fresh and preserved juvenile and adult specimens of the Atlantic *Gymnura* species complex by region. Measurement mean and range expressed as percentage of disk width (DW) unless otherwise indicated; samples size in parentheses. Regional coverage: Western North Atlantic (Delaware to Florida, USA); Gulf of Mexico (Florida to Texas, USA); Suriname (Venezuela, Suriname, French Guiana); Eastern Atlantic (Mauritania, Senegal, Guinea-Bissau, Guinea, Sierra-Leone, Liberia, Côte d'Ivoire, Ghana, Togo, Benin, Nigeria, Angola). Abbreviations for morphometrics provided in Figure 2.

	Male				**Female**			
	Western North Atlantic (23)	Gulf of Mexico (57)	Suriname (24)	Eastern Atlantic (14)	Western North Atlantic (36)	Gulf of Mexico (53)	Suriname (22)	Eastern Atlantic (15)
LT (mm)	253.7	263.4	219.4	259.2	400.7	382.8	254.6	325.9
	150-372	100-375	126-190	151-389	133-633	136-642	132-420	130-544
DW (mm)	342.1	343.4	278.9	339.4	557.3	514.2	360.5	468.3
	222-487	159-459	170-351	202-525	210-870	208-838	184-582	179-750
TL (mm)	52.7	56.8	55.3	66.8	89.1	92.7	59.7	81.1
	32-80	11 to 90	36-77	41-103	22-150	27-169	30-101	34-139
LAP	62.1	63.8	62.2	61.1	60.4	60.4	59.5	59.4
	55.5-66.9	59.5-69.5	57.2-69.2	58.6-63.4	57.7-63.2	42.9-63.4	55.4-62.0	57.0-61.1
LPP	50.6	50.5	50.5	50.7	51.2	50.8	50.4	50.5
	47.7-53.4	46.5-53.5	45.5-53.2	49.0-52.6	49.6-53.3	47.4-53.8	49.2-53.0	48.0-53.5
LB	59.6	62.6	60.7	56.9	57.9	58.0	54.6	54.6
	52.0-66.7	53.2-67.3	52.3-68.5	53.5-60.1	51.4-62.1	53.4-61.9	53.2-56.5	52.6-58.5
LD	58.2	60.4	58.5	56.1	56.3	56.2	54.0	54.3

	51.1-64.9	50.9-68.4	51.4-65.1	53.0-59.2	52.0-59.3	51.3-58.9	52.0-55.8	52.4-56.8
LH	18.6	20.9	19.6	18.1	18.2	16.6	16.6	16.4
	15.9-22.3	15.9-24.3	16.6-22.5	16.9-19.2	13.6-64.6	14.2-19.0	15.0-19.4	13.0-19.1
LPOBS	11.6	12.9	11.4	10.1	9.8	9.3	8.8	8.9
	9.7-14.8	8.7-15.9	8.8-14.2	8.7-12.5	8.9-10.6	7.7-11.3	8.1-9.6	8.1-9.5
WIO	8.7	8.8	8.6	9.6	8.7	8.6	8.2	9.2
	7.8-11.3	7.6-11.1	7.9-9.7	9.1-10.3	7.7-9.8	7.7-10.3	7.3-8.7	8.5-9.7
WIS	9.4	9.7	8.9	9.3	9.2	9.1	8.6	8.8
	8.7-10.5	8.8-11.1	8.3-9.7	8.7-10.3	8.4-9.8	8.4-10.2	7.8-9.7	7.9-9.7
LSV	52.7	53.8	51.8	48.8	52.2	49.9	46.4	46.5
	44.5-71.1	45.9-64.9	46.6-58.3	45.2-52.5	45.9-84.4	46.4-52.9	45.1-48.1	44.6-50.0
LSG1	17.8	19.1	17.7	16.9	15.2	14.9	15.1	15.0
	16.1-21.3	15.8-22.5	10.3-21.5	15.3-18.1	14.0-16.3	13.8-17.0	13.8-17.3	14.1-17.2
DG5	11.3	12.1	10.8	11.8	11.8	12.1	10.6	11.4
	10.2-13.5	10.6-16.1	9.4-12.2	11.0-13.4	10.4-15.7	11.0-16.8	9.1-11.4	10.4-12.3
DG1	15.7	16.4	16.0	17.4	16.4	16.4	16.3	17.2
	14.4-18.4	14.4-27.0	14.7-17.5	16.8-19.4	14.5-17.7	11.8-18.2	15.3-18.0	16.4-19.6
LAPV	7.8	8.2	8.8	7.3	7.2	7.4	6.9	6.7
	6.6-9.2	5.6-9.6	6.9-11.5	5.8-9.1	5.5-8.8	5.2-11.7	5.9-7.6	5.5-7.6
SP	7.5	7.4	7.0	7.2	8.3	7.8	6.9	7.6
	6.4-8.6	6.1-9.0	6.0-8.1	6.7-7.7	6.5-9.6	6.2-9.7	5.9-8.0	6.2-9.7
LPN	10.4	11.6	10.4	9.0	8.4	8.1	8.0	7.9
	8.7-13.6	8.0-14.2	8.0-12.8	8.1-9.8	7.7-9.1	6.3-9.8	7.4-8.8	7.2-8.6
LPOLS	12.6	14.0	12.7	11.1	10.3	10.0	9.8	9.9
	10.7-15.5	10.0-16.7	10.1-15.9	10.0-12.0	9.2-11.1	8.7-11.4	9.0-10.7	8.5-13.3
LNC	1.8	2.1	1.6	1.8	1.7	1.6	1.4	1.6
	1.3-2.5	1.5-2.5	1.2-2.1	1.5-2.1	1.4-1.9	1.2-2.0	1.1-1.7	1.5-1.9
WIN	6.2	6.8	6.3	6.5	6.3	6.5	5.9	6.2
	5.7-7.0	6.0-8.4	5.5-7.3	6.0-7.0	5.5-7.0	6.0-8.2	5.5-6.4	5.4-7.4
WNC	7.7	8.2	7.3	7.9	7.6	7.6	6.9	7.3

	6.8-9.1	6.4-10.0	6.6-8.2	7.3-8.4	6.5-8.7	7.1-8.6	6.1-8.6	6.2-8.0
WM	8.7	9.3	8.6	8.8	9.1	9.3	8.5	8.3
	8.0-10.2	8.4-10.5	7.9-9.1	7.8-9.9	7.8-10.1	8.1-10.4	7.9-9.2	7.7-9.4
OLCL	5.0	5.5	3.9	3.5				
	2.1-7.4	1.7-7.6	1.5-6.4	2.0-5.2				
ILCL	9.1	10.3	8.2	6.3				
	3.0-13.1	5.3-12.8	4.7-13.5	4.0-8.9				
TL % LT	20.8	21.7	25.1	26.2	21.5	23.7	23.4	25.0
	17.6-24.9	11.0-26.3	23.0-29.4	22.1-28.8	14.2-25.6	14.5-27.2	15.0-26.6	22.7-26.5
LPP % LAP	81.7	79.2	81.2	83.2	84.9	83.9	84.7	85.2
	74.1-92.1	72.5-87.6	75.2-87.3	79.6-87.2	79.4-90.4	76.2-91.6	79.3-92.9	79.1-92.0
WIN % LPN	60.3	59.1	60.8	72.4	75.2	80.0	74.3	78.2
	49.7-68.8	47.5-80.8	49.1-72.5	66.0-82.5	62.4-87.2	67.3-100.0	67.6-82.7	72.0-89.9
LNC % LPOLS	14.2	14.9	12.8	16.2	16.5	16.2	14.0	16.6
	11.1-18.6	12.0-18.8	10.0-16.5	13.4-19.8	12.9-19.7	11.5-19.8	11.9-16.5	11.9-18.9
SP % DG1	48.0	45.0	43.7	41.0	50.6	48.1	42.2	44.1
	38.1-56.2	29.3-51.9	37.9-51.8	38.4-43.6	37.9-60.6	36.6-66.9	36.5-48.1	35.6-56.1
DW:LD	1.7	1.7	1.7	1.8	1.8	1.8	1.9	1.8
	1.5-2.0	1.5-2.0	1.5-1.9	1.7-1.9	1.7-1.9	1.7-2.0	1.8-1.9	1.8-1.9

Figures

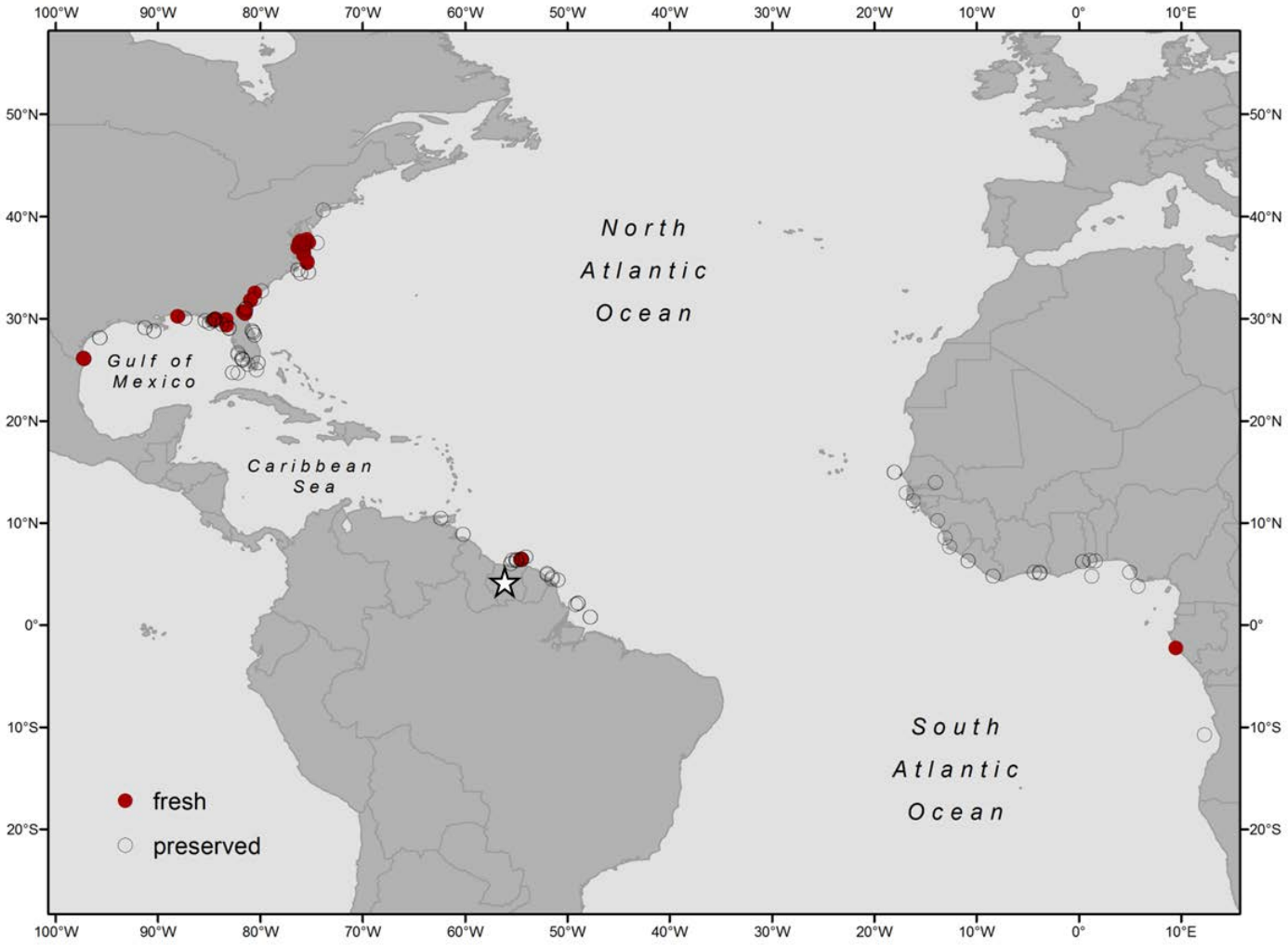

100°W
90°W
80°W
70°W
60°W
50°W
40°W
30°W
20°W
10°W
0°
10°E
50°N
40°N
30°N
20°N
10°N
0°
10°S
20°S
North
Atlantic
Ocean
Gulf of
Mexico
Caribbean
Sea
South
Atlantic
Ocean
fresh
preserved

FIGURE 1. Map of North America, South America, and Africa locations of fresh (filled circle) and preserved (open circle) *Gymnura* specimens used for morphometric and genetic analysis. The type locality (Suriname, South America) for *Gymnura micrura* is indicated by the star.

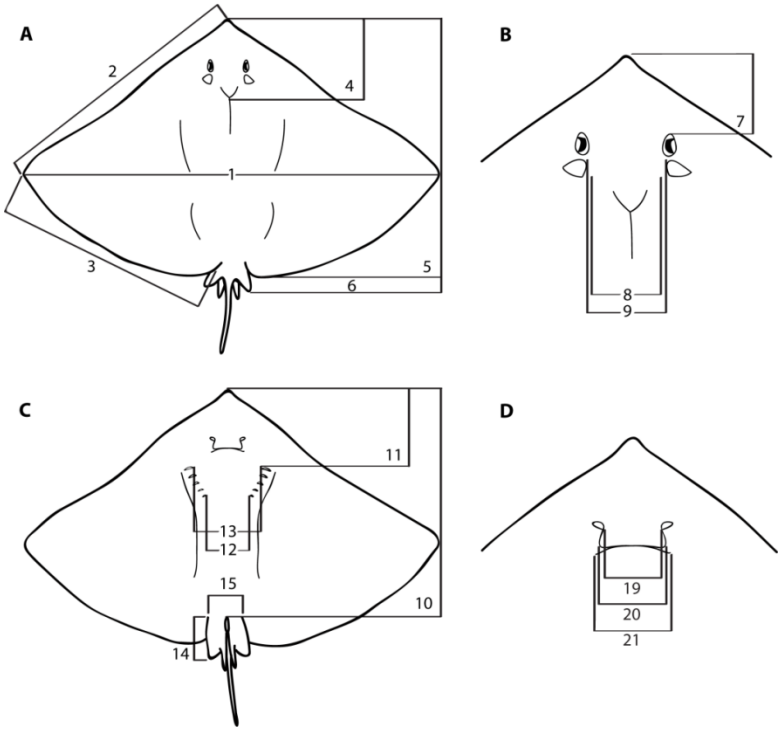


FIGURE 2. Morphometric characters and abbreviations used in this study, adapted from Smith *et al*. 2009. (a) 1, disc width (DW); 2, anterior pectoral length (LAP); 3, posterior pectoral length (LPP); 4, body length (LB); 5, disc length (L_D); 6, head length (LH). (b) 7, pre-orbital snout length (LPOBS); 8, inter-orbital width (WIO); 9, interspiracular width (WIS). (c) 10, snouft to vent length (LSV); 11, snout to first gill length (LSG1); 12, fifth gill transverse distance (DG5); 13, first gill transverse distance (DG1); 14, anterior pelvic length (LAPV); 15, pelvic span (SP). (d) 16, pre-narial length (LPN); 17, pre-oral snout length (LPOLS); 18, nasal curtain length (LNC); 19, inter-narial width (WIN); 20, nasal curtain width (WNC); 21, mouth width (WM).

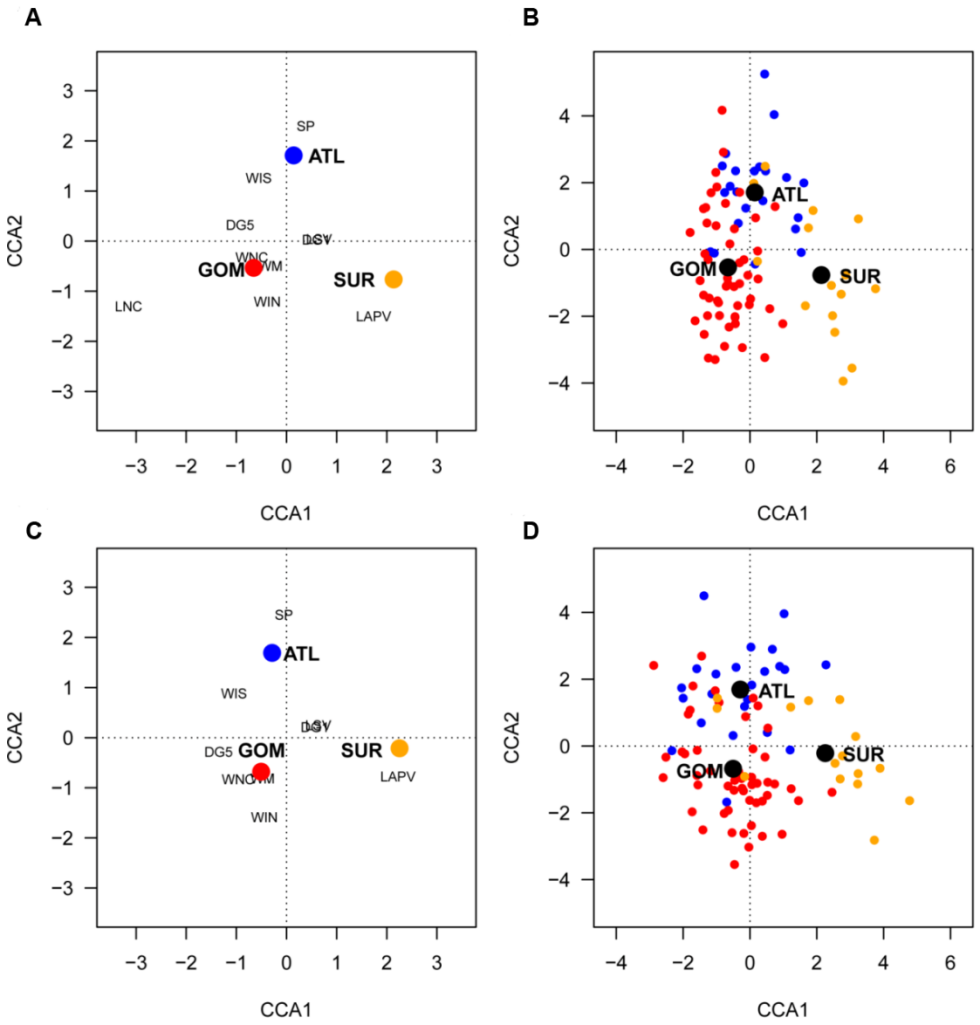


FIGURE 3. Canonical Correlation Analysis plots of 10 morphometric characters (WIS, LSV, DG5, DG1, LAPV, SP, LNC, WIN, WNC, WM) of juvenile and adult male specimens of the *Gymnura* complex from the western North Atlantic (ATL), Gulf of Mexico (GOM), and Suriname (SUR – including Venezuela and French Guiana). The first canonical axis (CA1) and CA2 accounted for 80% and 20% of the variation explained, respectively, and LNC and SP contributed most to differences between geographic regions (a) and individual variability (b). The proportion of variation explained without LNC was 73% and 27% for CA1 and CA2, respectively, and significant regional separation of specimens was retained by the remaining nine characters (c, d).

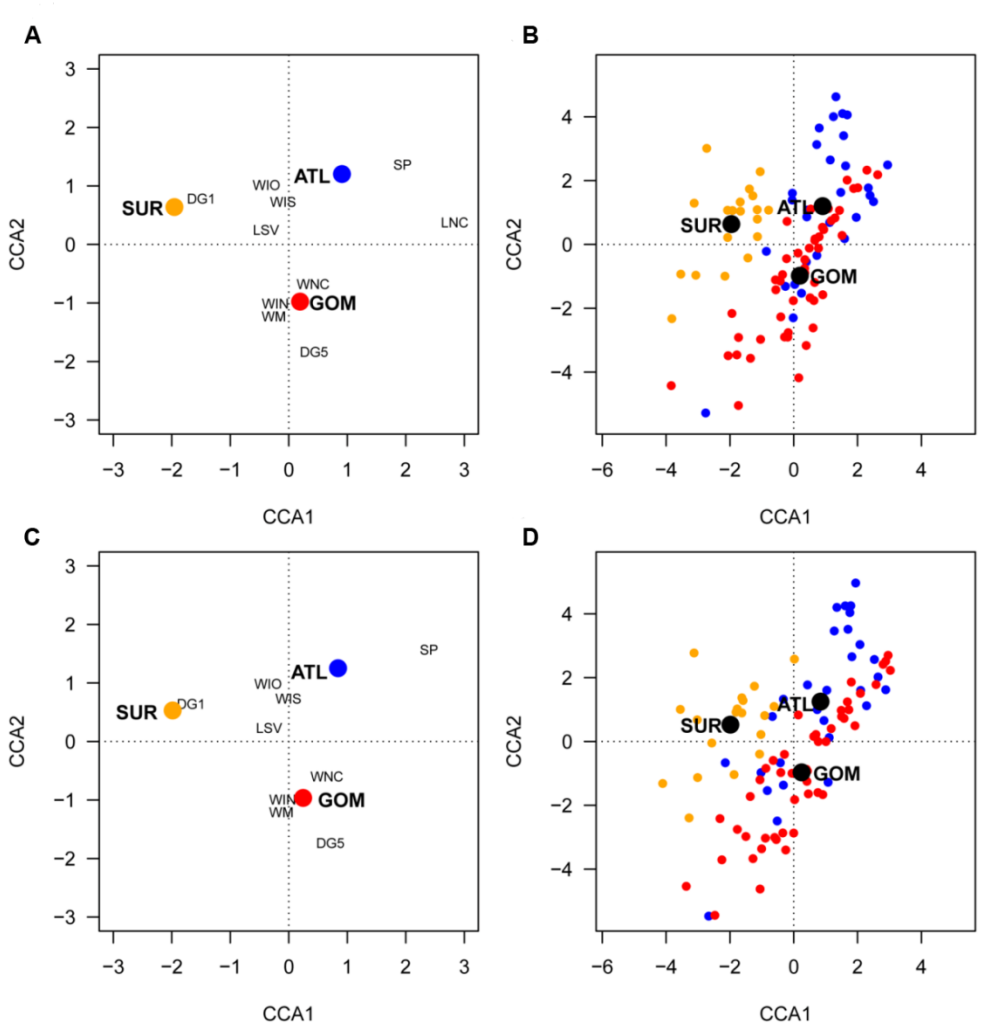


FIGURE 4. Canonical Correlation Analysis plots of 10 morphometric characters (WIO, WIS, LSV, DG5, DG1, SP, LNC, WIN, WNC, WM) of juvenile and adult female specimens of the *Gymnura* complex from the western North Atlantic (ATL), Gulf of Mexico (GOM), and Suriname (SUR – including Venezuela and French Guiana). The first canonical axis (CA1) and CA2 accounted for 78% and 22% of the variation explained, respectively, and LNC and SP contributed most to differences between geographic regions (a) and individual variability (b). The proportion of variation explained without LNC was 71% and 29% for CA1 and CA2, respectively, and significant regional separation of specimens was retained by the remaining nine characters (c, d).

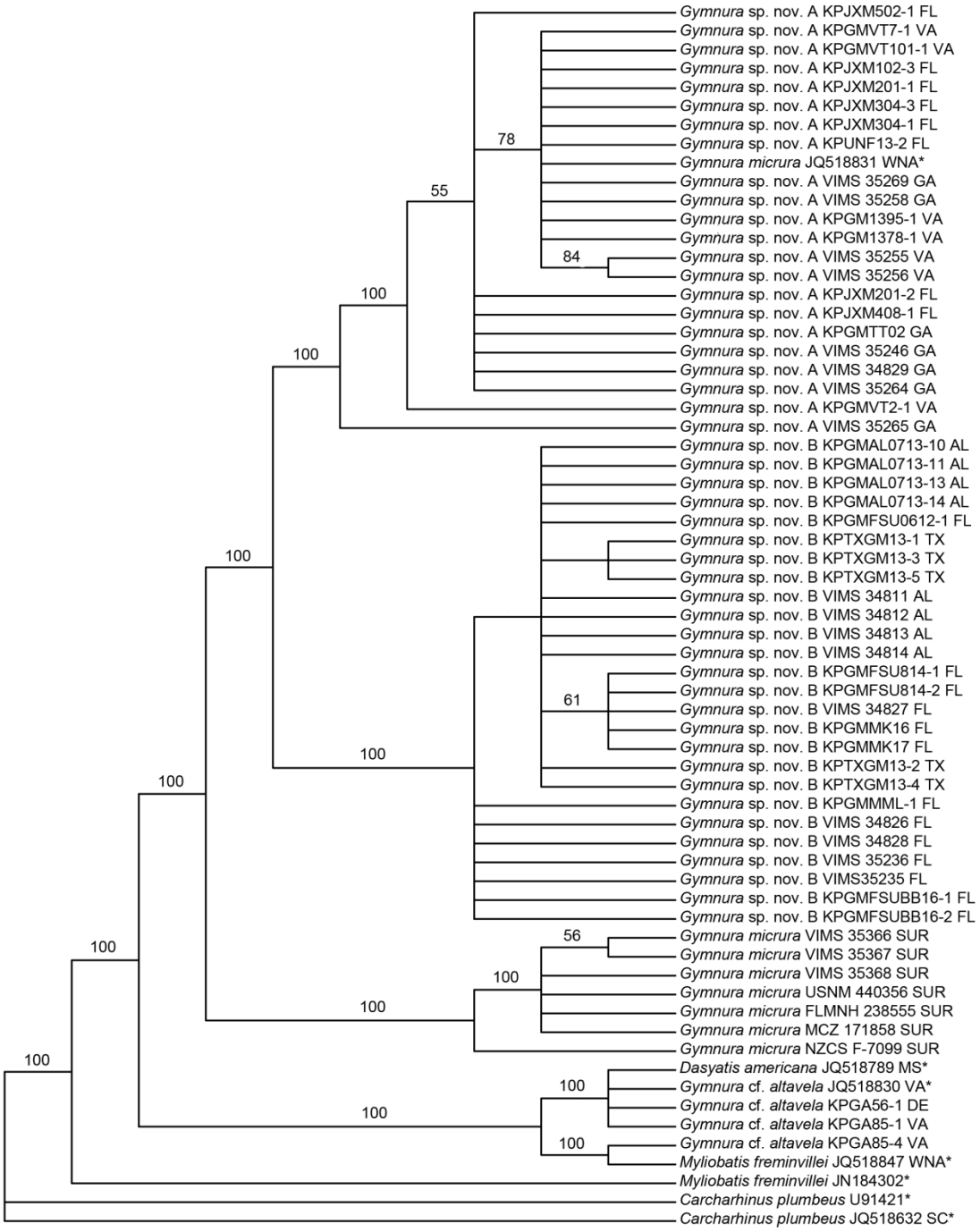
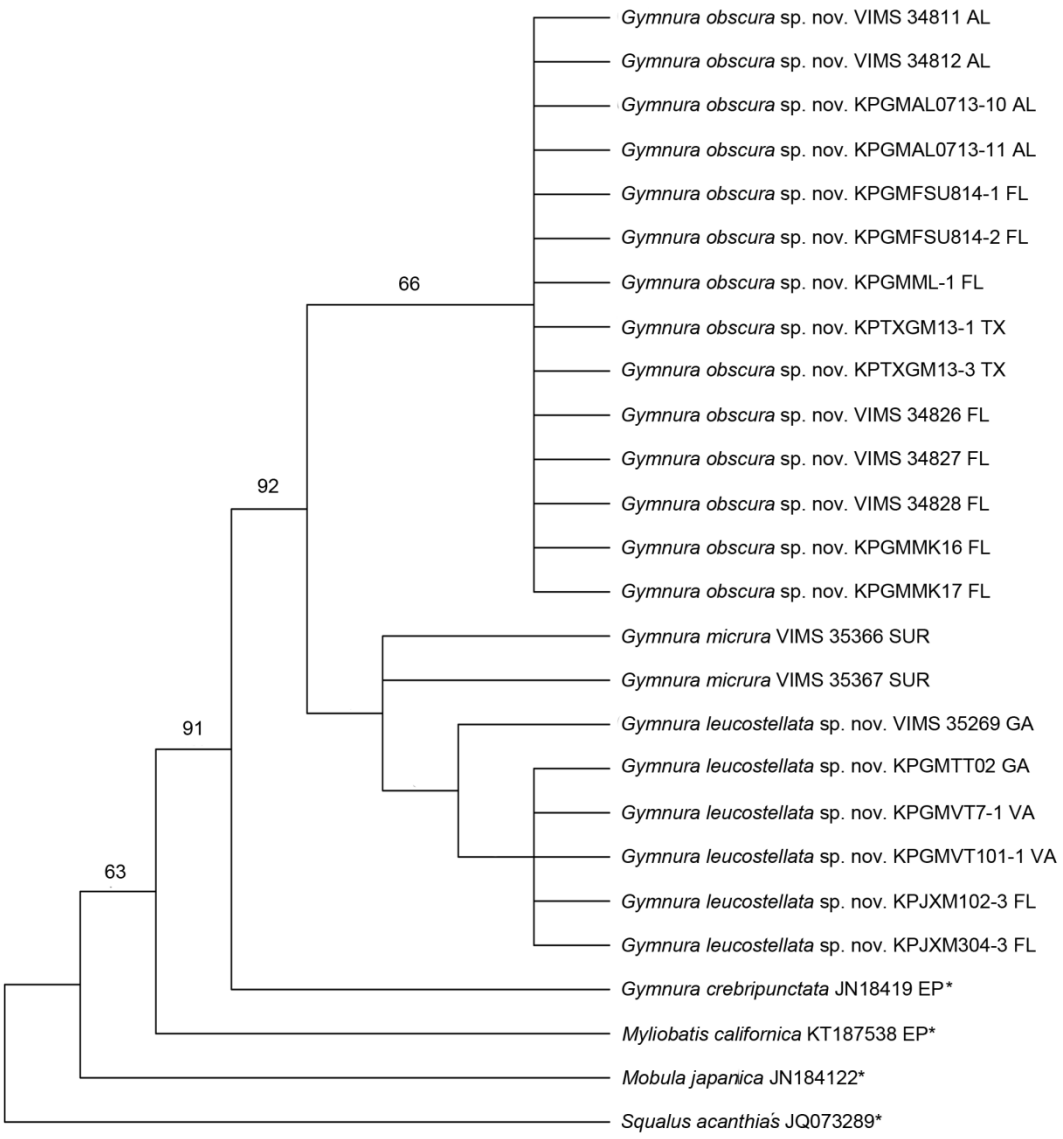


FIGURE 5. Majority rule bootstrap consensus tree of mitochondrial ND2 sequences for 67 taxa, including the outgroup shark *Carcharhinus plumbeus.* Specimen localities are abbreviated: DE – Delaware; VA – Virginia; SC – South Carolina; GA – Georgia; FL – Florida; AL – Alabama; MS – Mississippi; TX – Texas; SUR – Suriname; GAB – Gabon; SEN – Senegal; WNA – western North Atlantic; EP – East Pacific. Data from GenBank indicated by *.


Gymnura obscura sp. nov. VIMS 34811 AL
Gymnura obscura sp. nov. VIMS 34812 AL
Gymnura obscura sp. nov. KPGMAL0713-10 AL
Gymnura obscura sp. nov. KPGMAL0713-11 AL
Gymnura obscura sp. nov. KPGMFSU814-1 FL
Gymnura obscura sp. nov. KPGMFSU814-2 FL
66
Gymnura obscura sp. nov. KPGMML-1 FL
Gymnura obscura sp. nov. KPTXGM13-1 TX
Gymnura obscura sp. nov. KPTXGM13-3 TX
Gymnura obscura sp. nov. VIMS 34826 FL
Gymnura obscura sp. nov. VIMS 34827 FL
92
Gymnura obscura sp. nov. VIMS 34828 FL
Gymnura obscura sp. nov. KPGMMK16 FL
Gymnura obscura sp. nov. KPGMMK17 FL
Gymnura micrura VIMS 35366 SUR
Gymnura micrura VIMS 35367 SUR
91
Gymnura leucostellata sp. nov. VIMS 35269 GA
Gymnura leucostellata sp. nov. KPGMTT02 GA
Gymnura leucostellata sp. nov. KPGMVT7-1 VA
Gymnura leucostellata sp. nov. KPGMVT101-1 VA
63
Gymnura leucostellata sp. nov. KPJXM102-3 FL
Gymnura leucostellata sp. nov. KPJXM304-3 FL
Gymnura crebripunctata JN18419 EP*
Myliobatis californica KT187538 EP*
Mobula japanica JN184122*
Squalus acanthias JQ073289*

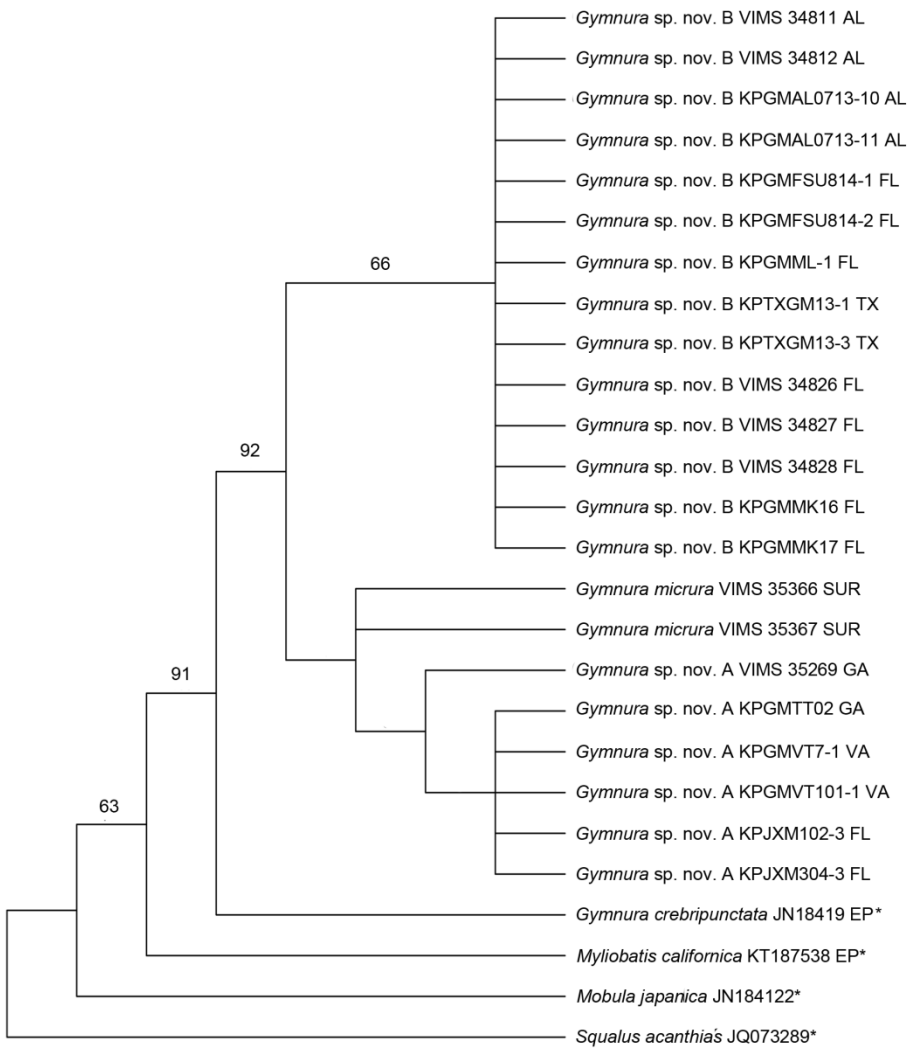


FIGURE 6. Majority rule bootstrap consensus tree of nuclear RAG-1 sequences for 27 taxa, including the outgroup shark *Squalus acanthias.* Specimen localities are abbreviated: VA – Virginia; GA – Georgia; FL – Florida; AL – Alabama; TX – Texas; SUR – Suriname; GAB – Gabon; EP – East Pacific. Boostrap support values are indicated at branch nodes. Data from GenBank indicated by *.

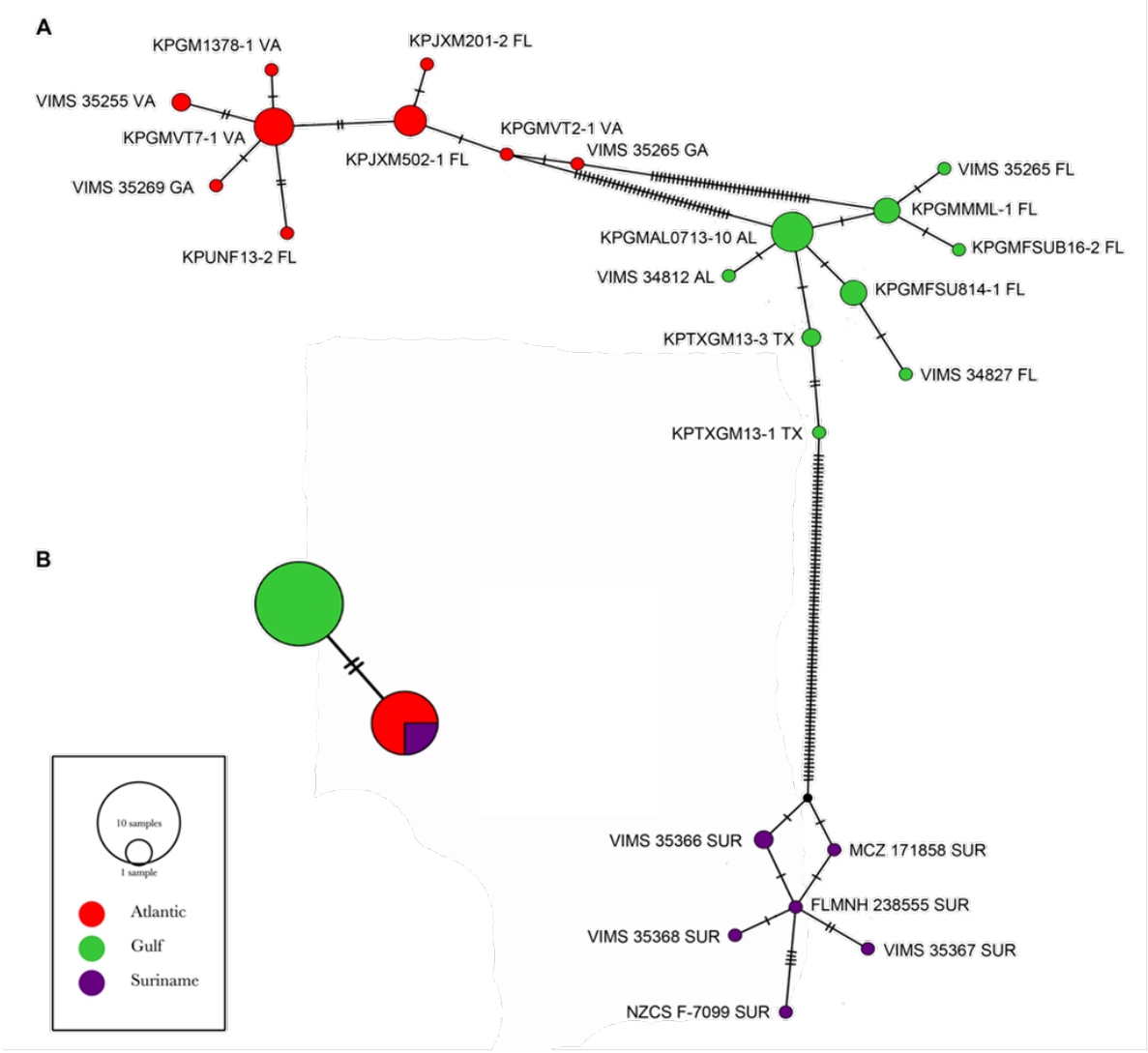


FIGURE 7. Median joining haplotype networks of mitochondrial ND2 (a) and nuclear RAG-1 (b) sequences from western North Atlantic *Gymnura* sp. nov. A (red), Gulf of Mexico *Gymnura* sp. nov. B (green), and Suriname *G. micrura* (purple). Branch lengths correspond to the magnitude of genetic divergence between sequences, and hash marks indicate the number of nucleotide differences. Specimen locality abbreviations are provided in Figure 5.

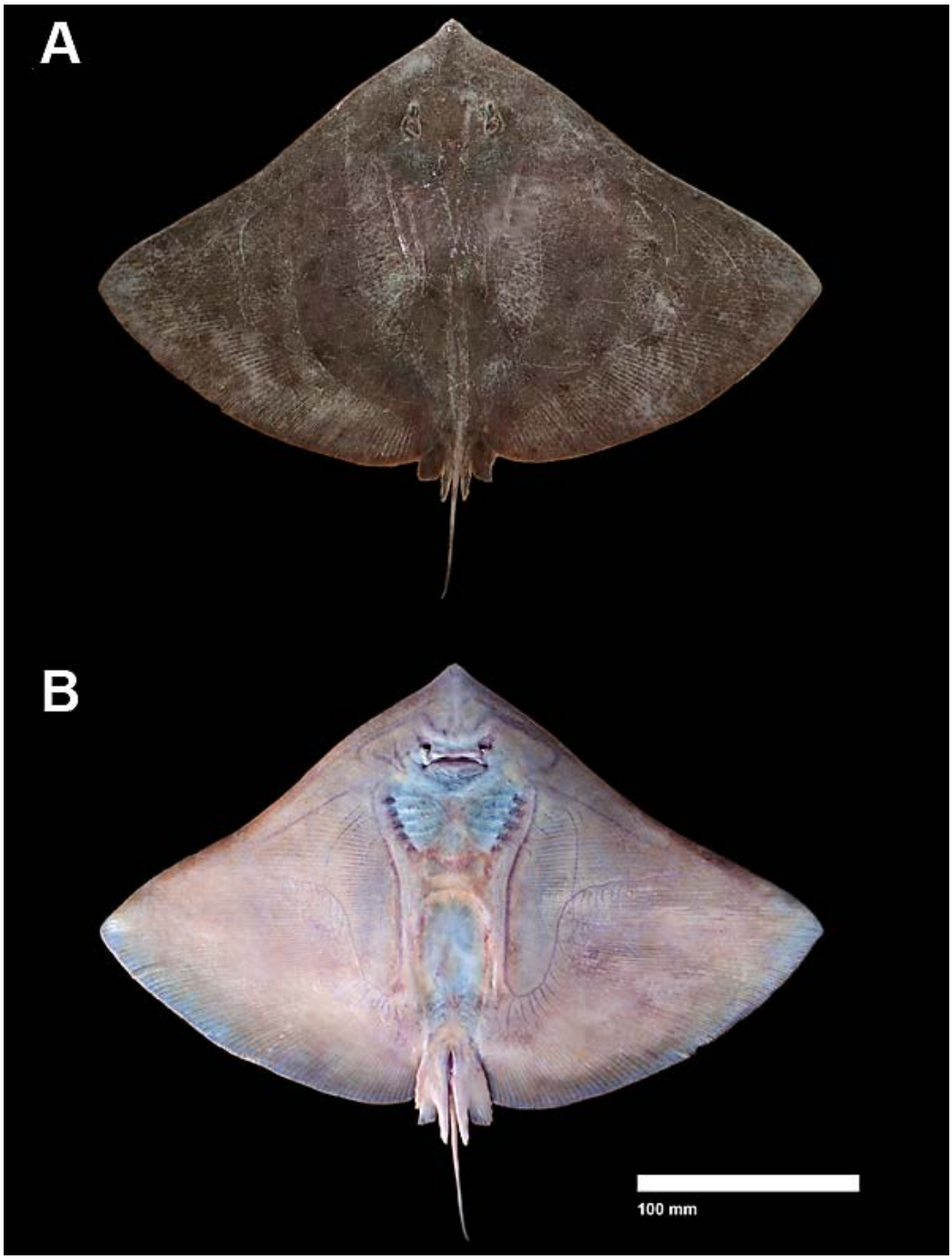


FIGURE 8. Dorsal (A) and ventral (B) view of *Gymnura micrura* neotype USNM 440357, adult male 330 mm DW, Suriname, South America.

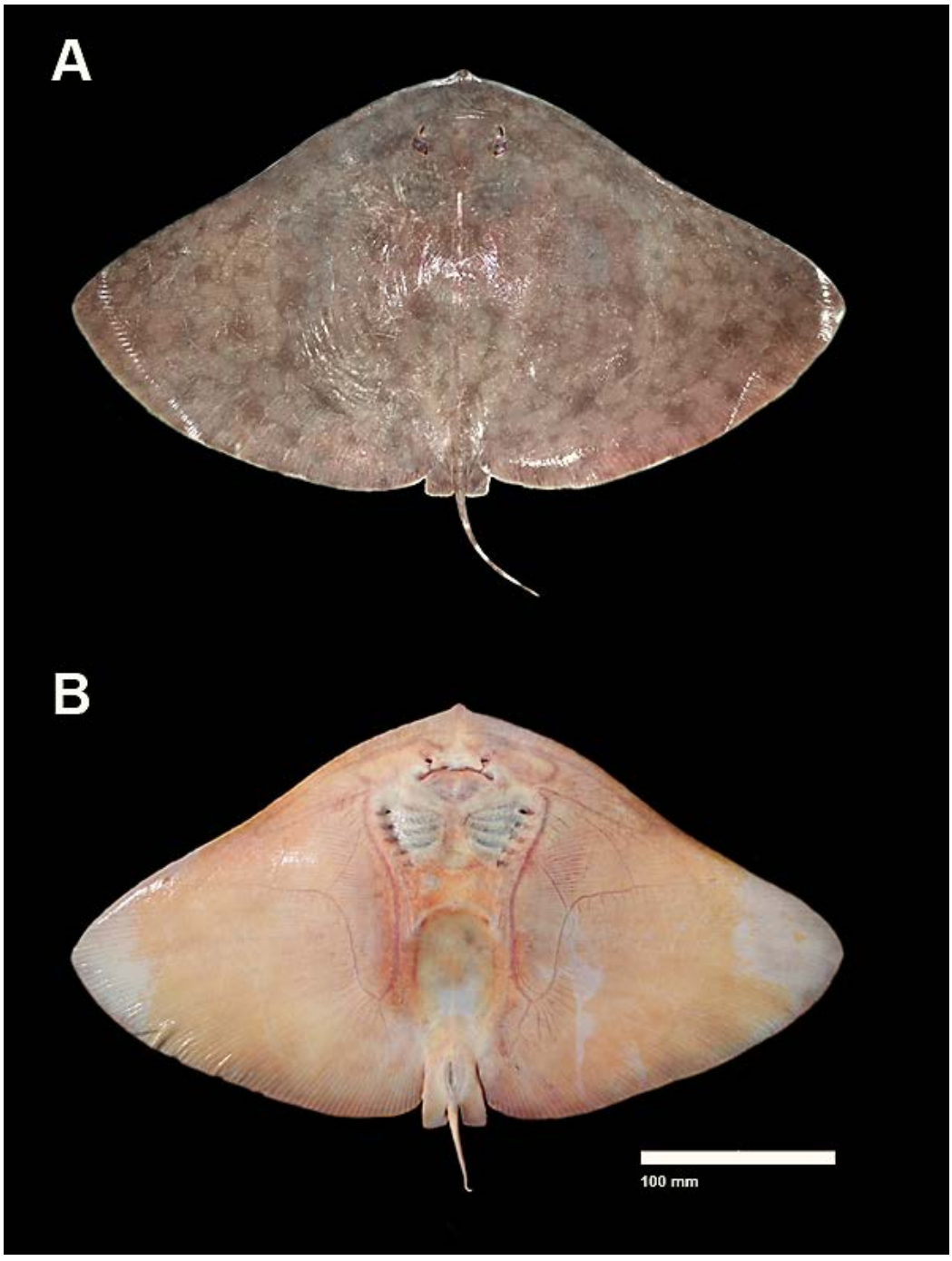


FIGURE 9. Dorsal (A) and ventral (B) view of *Gymnura micrura* paratype USNM 440356, adult female, 582 mm DW, Suriname, South America.

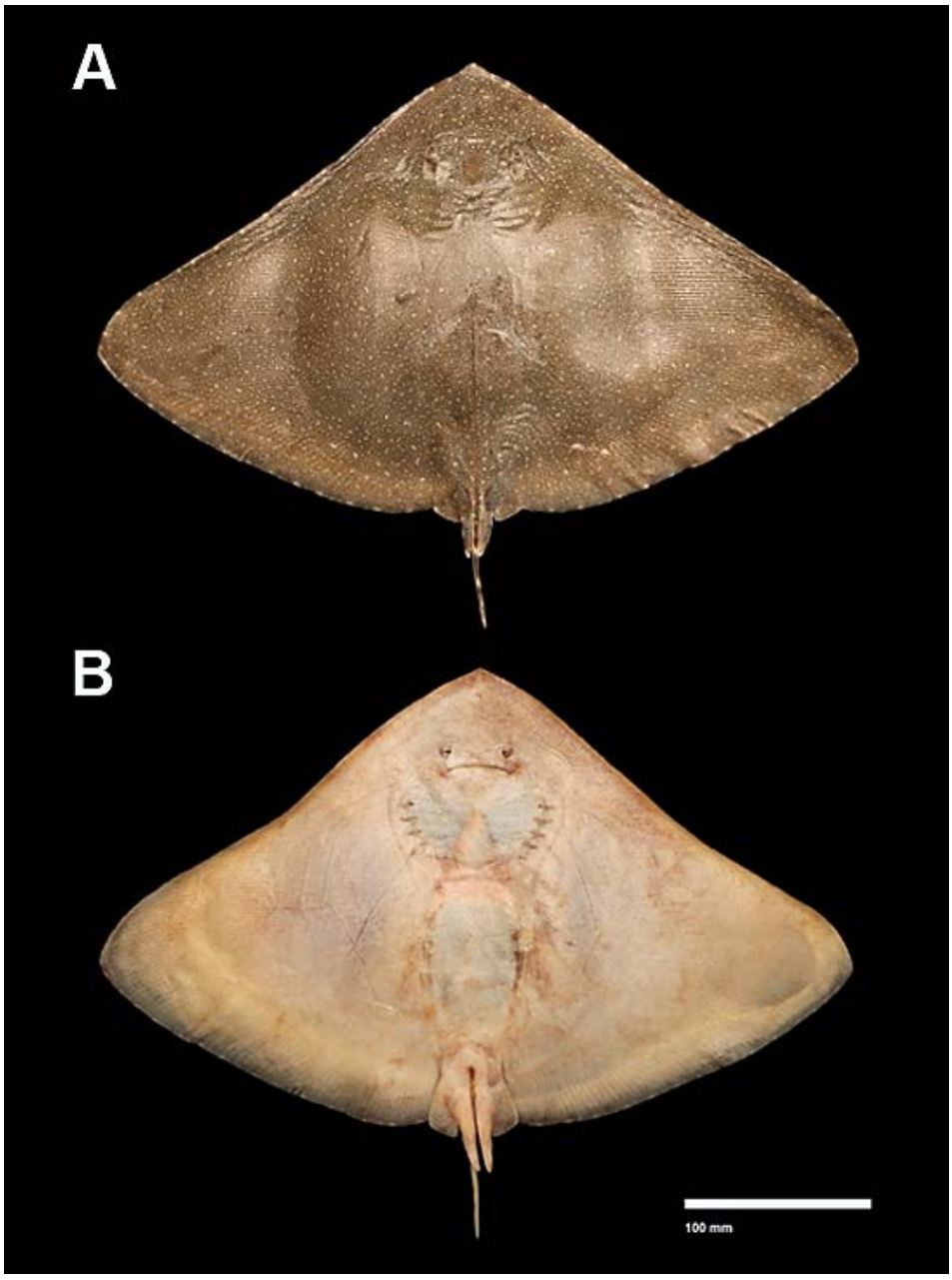


FIGURE 10. Dorsal (A) and ventral (B) view of *Gymnura* sp. nov. A holotype USNM 440358, adult male 406 mm DW, North Carolina, USA.

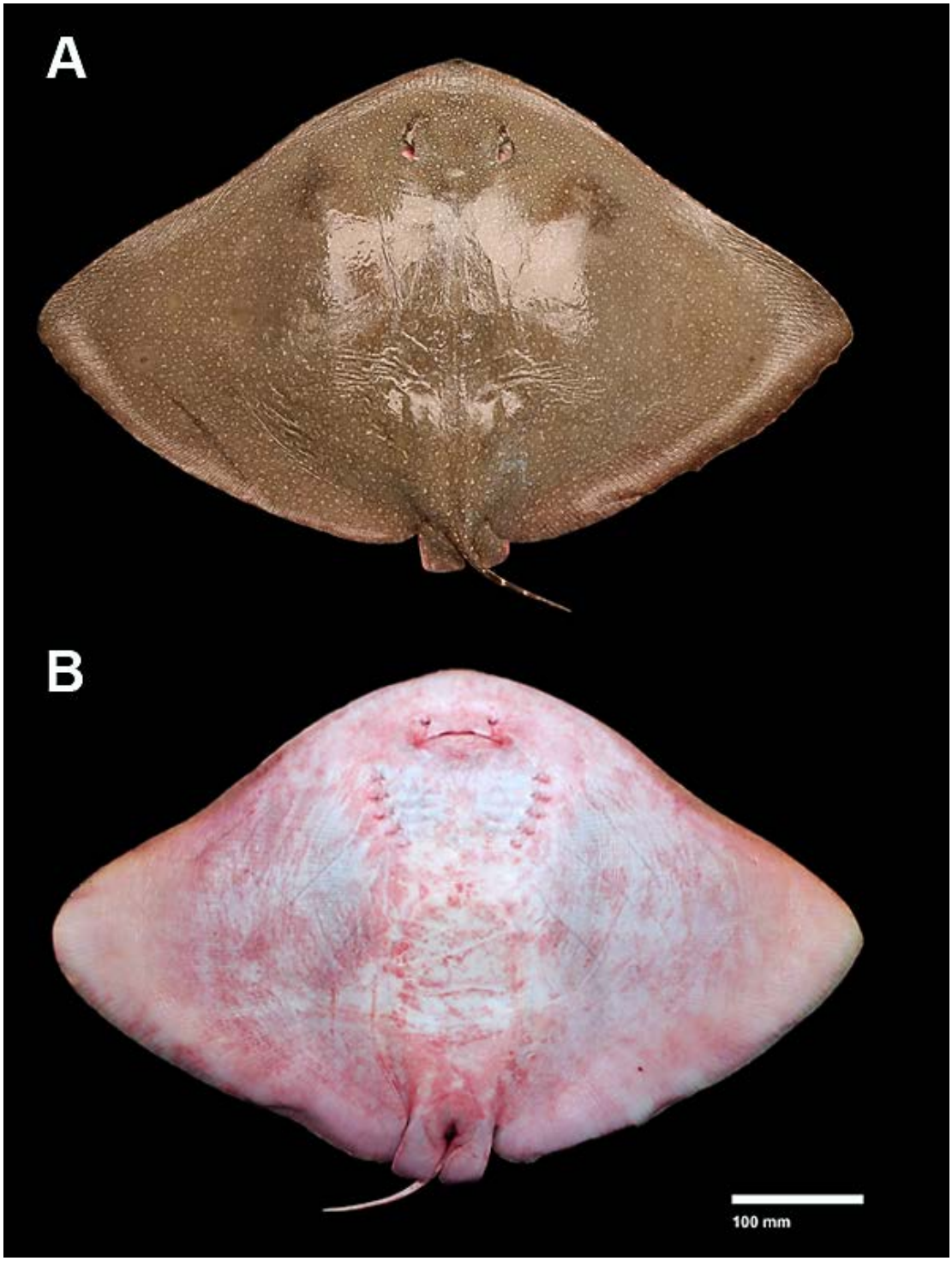


FIGURE 11. Dorsal (A) and ventral (B) view of *Gymnura* sp. nov. A paratype USNM 440359, adult female, 638 mm DW, Georgia, USA.

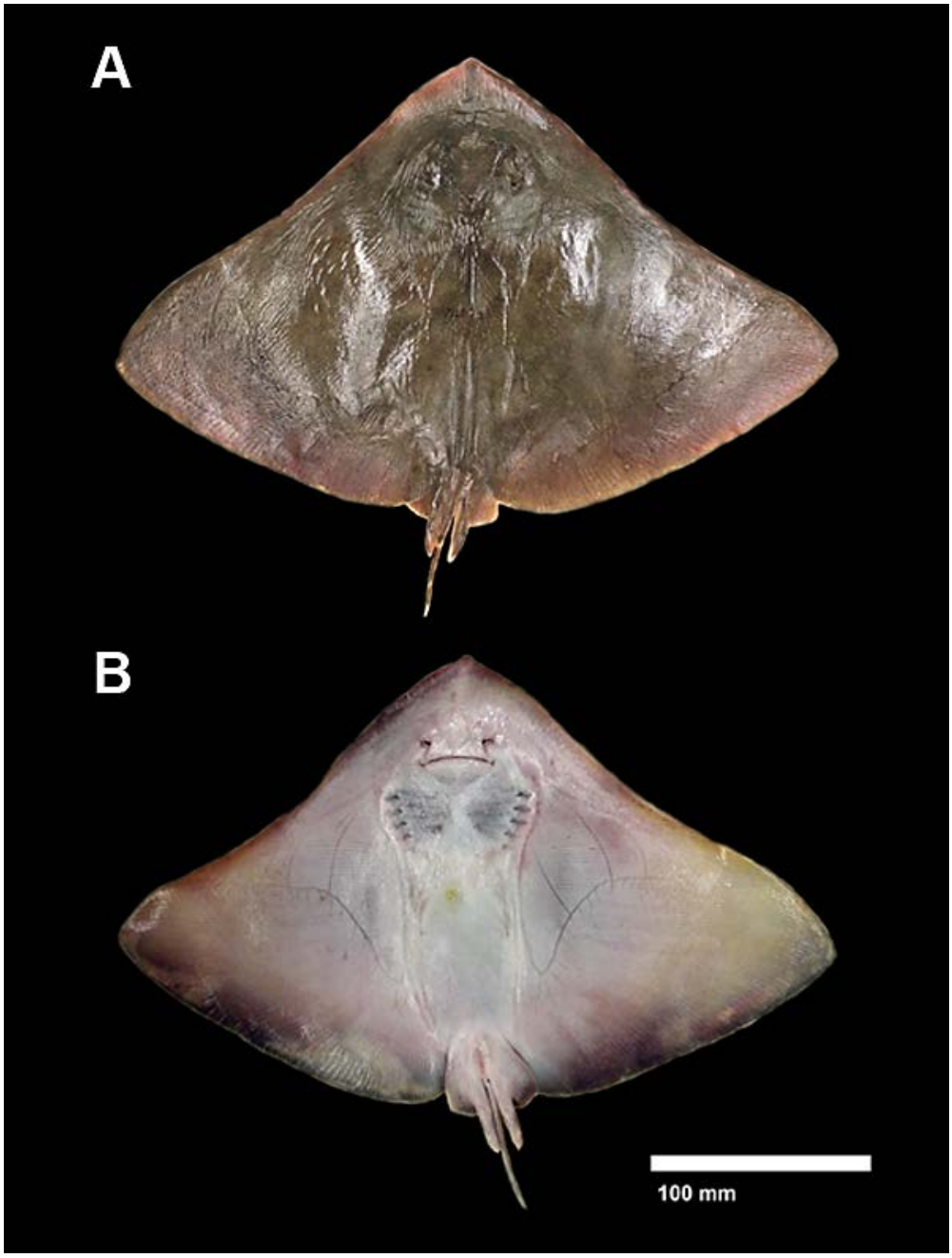


FIGURE 12. Dorsal (A) and ventral (B) view of *Gymnura* sp. nov. B holotype USNM 440360, adult male, 331 mm DW, Florida, USA.

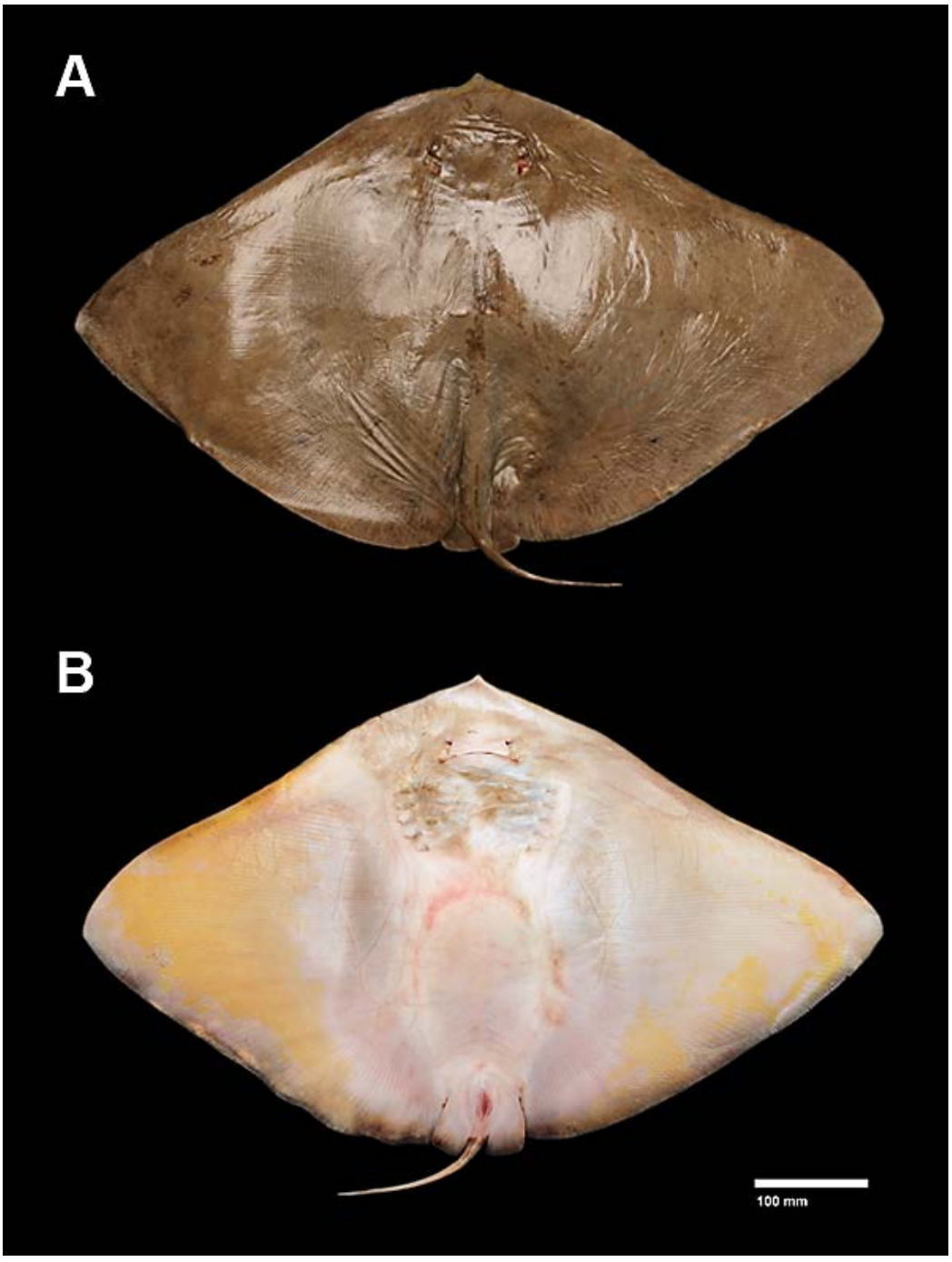


FIGURE 13. Dorsal (A) and ventral (B) view of *Gymnura* sp. nov. B paratype USNM 440361, adult female 694 mm DW, Alabama, USA.

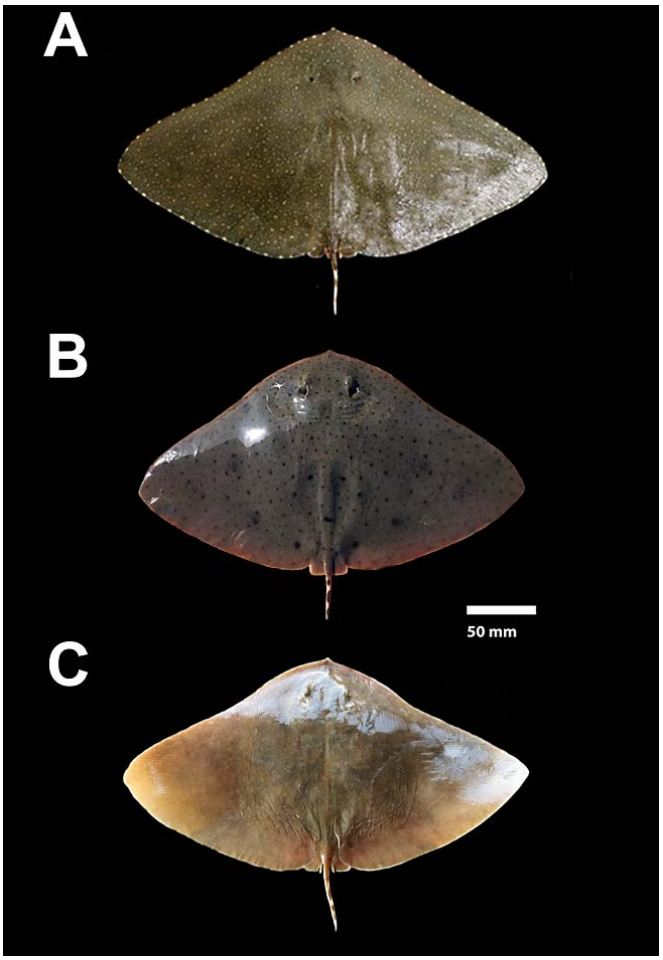


FIGURE 14. Variation in disk coloration and patterns between (a) juvenile male *Gymnura* sp. nov. A, Florida east coast (b) juvenile female Gulf of Mexico *Gymnura* sp. nov. B, Florida west coast, and (c) juvenile male *Gymnura* sp. nov. B, Alabama. Panals a and c are not to scale.

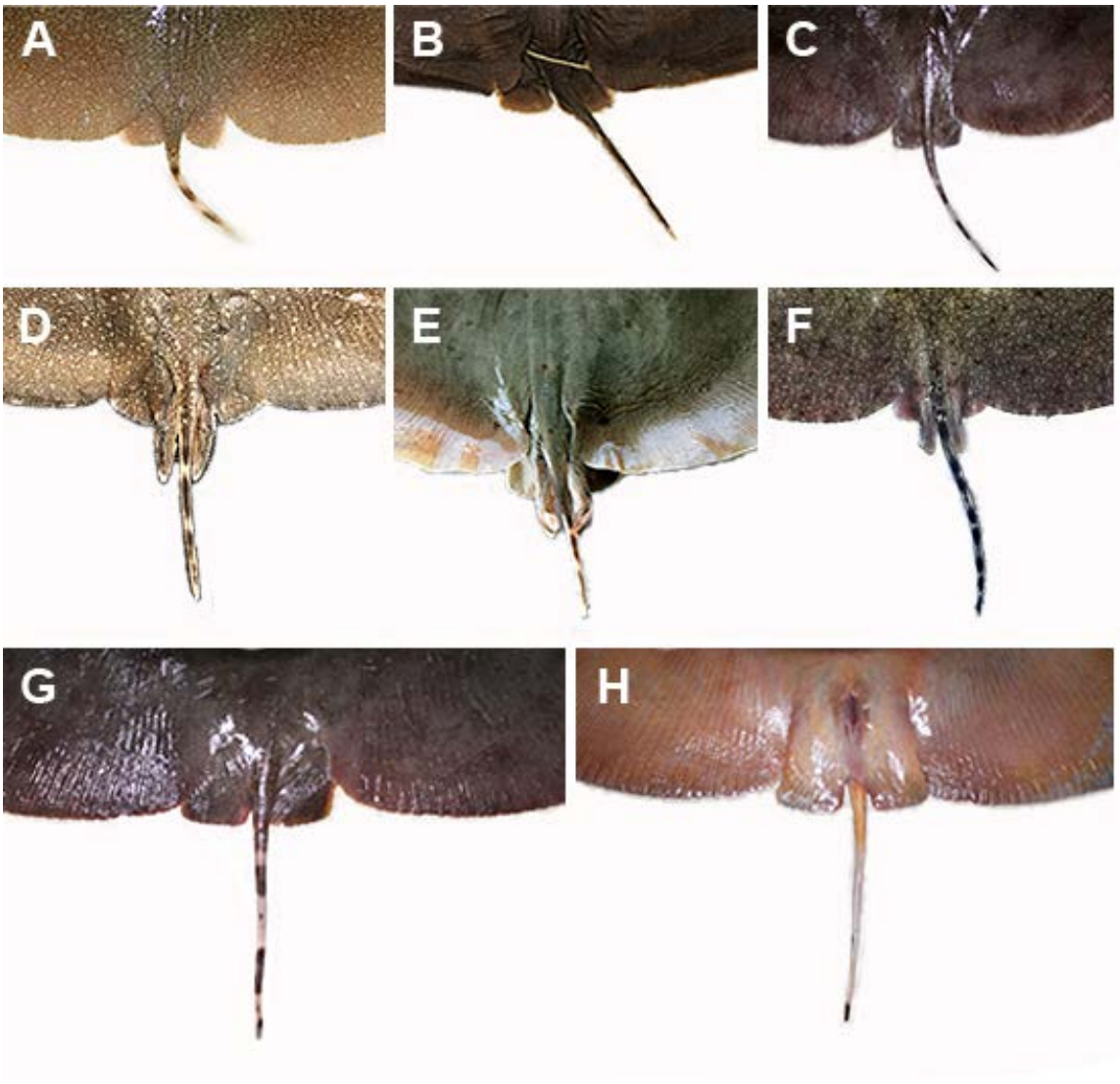


FIGURE 15. Ontogenetic and geographical variation in Atlantic *Gymnura* tail morphology and coloration: *Gymnura* sp. nov. A (a) young-of-year and (b) adult female; Suriname *G. micrura* adult female (c); *Gymnura* sp. nov. A adult male (d); *Gymnura* sp. nov. B adult male (e); Gabon *G. micrura* adult male (f); Suriname *G. micrura* female dorsal (g) and ventral view (h).

CHAPTER 5

CONCLUSIONS

CONCLUSIONS

Summary

This dissertation was designed to address the generally poor understanding of U.S. batoid populations in the western North Atlantic through life history and taxonomic investigations of a common and widely distributed family of rays, the Gymnuridae. In order to achieve the objectives of each chapter, adequate empirical data from *Gymnura altavela* and *G. micrura* specimens collected throughout their range of distribution, across ontogeny, and throughout the year was necessary to answer biological questions at the species and population levels. Due to their broad U.S. distribution (New York to Texas), undocumented seasonal migration and movement patterns, and large body sizes, specimen acquisition was dependent on a broad-scale collaboration between a network of agencies, institutions, and laboratories. Notably, fishery-independent survey programs were key to obtaining sufficient data to accomplish research goals, reflecting the valuable but underutilized resource that such programs offer. This approach provided data used in Chapters 2 and 3 to describe age and growth patterns, size and timing of maturation, seasonality of critical reproductive stages, embryonic development, and fecundity for three Gymnuridae in the western North Atlantic Ocean. While rough life history parameter estimates and reproductive biology have been previously reported in the literature, this dissertation provides improved estimates based on a broader spatiotemporal sampling of populations, sexes, and life stages occurring in U.S. waters. Knowledge of the patterns of growth, the time required to reach reproductive maturity, and the reproductive capacity (i.e. lifetime fecundity) of female rays can directly be used by assessors to update the status of *Gymnura* populations, and to identify critical research areas to prioritize in the future.

Improved understanding of the population dynamics of the batoid assemblage of the western North Atlantic relies on species-specific life history information for which accurate

species identification is fundamental. In Chapter 3, life history information for *G. micrura* differed between two adjacent regions along the U.S. coast, and provided the first indication of structuring in this population. Consistent geographic differences in body metrics including maximum size, maturity size, and fecundity were complimented by distinct morphologies of rays collected from the Mid-Atlantic region and from the northern Gulf of Mexico. These results lead to a pilot study conducted by William & Mary student intern Meredith Seitz, which revealed that inconsistencies in the biology and morphology of Atlantic and Gulf of Mexico rays were also present in the genetic structure of the species based on mitochondrial DNA analysis. A formal investigation into the taxonomy of *G. micrura* was undertaken in Chapter 4, and the contributions of Chapter 3 ultimately represent the first reports on the life history of two newly described species (*Gymnura* sp nov. A and *Gymnura* sp. nov. B) in U.S. waters. This dissertation can serve as a model for addressing the biological and taxonomic deficiencies in our knowledge of other batoid species using existing survey platforms and a multidisciplinary approach. Without accurate species identifications and basic life history information, the dynamics of these batoid populations cannot be fully understood and the vulnerability of species to extinction remains difficult to predict.

Relative to teleostean fishes, chondrichthyans including batoid species generally display slower growth to maturity and have lower annual fecundity (Fisher et al. 2013; Frisk 2010), which reduces lifetime productivity. Among batoids, the gymnurids demonstrate moderately fast growth rates over average lifespans based on the age and growth of *G. altavela*, however this species is the largest of the genus, and results presented here may therefore differ from the sympatric *Gymnura* sp. nov. A and other congeners. The oldest specimen in this study was conservatively aged to be 18 years old, and provides the first estimate of longevity for the entire family. Sexually-dimorphic changes were observed during ontogeny of *G. altavela*, with a shift from males and females of a similar size and shape during early life stages to males that reach

maximum size as females continue growing for several years. This pattern was also observed in the morphology of male and female *G. micrura* (i.e., *Gymnura* sp. nov. A and B). Sex-specific morphological differences associated with maturity were more extreme in these rays, with head shape becoming angular and more elongate in mature males, while female head shape remained broadly obtuse. While sexual dimorphism varies across batoid taxa, it has been reported for some other species of *Gymnura* (Bigelow and Schroeder 1953; Raje 2003; White and Dharmadi 2007; Jacobsen et al. 2009; Alkusairy et al. 2014), and is important to identify since such variable body shapes can lead to taxonomic confusion.

Life history information contained in this dissertation represent current populations of *G. altavela*, *Gymnura* sp. nov. A in the western North Atlantic Ocean, and *Gymnura* sp. nov. B in the northern Gulf of Mexico. Unfortunately, catch and abundance data for these populations are sparse, making inferences on the status of gymnurid species in U.S. waters challenging. Since *Gymnura* sp. nov. A grows to larger sizes, matures later, and has fewer offspring than *Gymnura* sp. nov. B, the risk of this species interacting with fishing gear before successfully reproducing is likely greater. Based on these results and generally declining trends in the abundance of gymnurids worldwide, a conservative approach to managing these species in U.S. waters is recommended, and efforts to improve monitoring are essential moving forward. Given that the state of research for many other U.S. batoid species is still in its infancy, a similar approach to their management is warranted until adequate species and population level data become available.

Future research and recommendations

There is much to be learned about the biology, ecology, and physiology of batoid fishes including the Gymnuridae. Age analyses demonstrated the utility of high resolution micro-computed tomography as an alternative method to visualize growth bands in *G. altavela*, although

this technique was not adequate for performing similar analyses on *Gymnura* sp. nov. A and B based on pilot study results (not presented here). Efforts to age the latter two species are needed to better understand growth patterns that may differ significantly from *G. altavela*, which grow nearly twice as large. Additionally, age estimates resulting from Chapter 2 require validation. For this study, I attempted to indirectly verify the seasonal periodicity of growth band formation through marginal increment analysis, however results were inconclusive due to inadequate monthly sampling of specimens. Validation of growth band formation using mark-recapture techniques should be explored in the near future, as this approach could also provide valuable data on seasonal movements and residency which have not been described for western North Atlantic gymnurids. During the summer, large and gravid *G. altavela* take up residency in the bays and inlets of the Virginia Eastern Shore and Chesapeake Bay (Vooren et al. 2007, J. Smith pers.comm.), providing the opportunity to conduct such studies. Investigations into the role that these Mid-Atlantic habitats play in the life cycle of *G. altavela* and other batoid species are needed, particularly if they represent critical nursery areas for early life stages.

Results presented in Chapter 3 on the reproductive biology of *G. altavela* could benefit from better temporal sampling of the population, since the timing and frequency of surveys limited the availability of specimens across all months. Reproductive periodicity and gestation cycles cannot be accurately described without monthly data on the condition of females. The frequency and abundance of this species along the U.S. east coast was low relative to *Gymnura* sp. nov. A, therefore a long term sampling effort across the species' range of distribution is needed. Furthermore, a taxonomic re-evaluation of this species is needed to determine if genetic differences reported between eastern and western Atlantic populations (Chapter 4) are indicative of a cryptic species present in U.S. waters (Naylor et al. 2012; Alkusairy et al. 2014).

Future research needs identified for *G. altavela*, including improved temporal sampling, habitat use, and migration studies are also warranted for *Gymnura* sp. nov. A and B. Additionally,

trends in the variation of reproductive characters across the family, such as gonad asymmetry, may provide insight on the systematics and evolutionary biology of the Gymnuridae. Therefore, histological analysis of the functionality of similar sized gonads observed in the newly described species (Chapter 3) is recommended.

For all three species, improved monitoring of populations and analysis of survey catch data is urgently needed. Data on seasonal and spatial occurrence, trends in abundance, and sex and life stage composition of catches is easily attainable from existing trawl surveys. Additionally, a multitude of studies across a variety of disciplines can be supported by these surveys to improve our understanding of western North Atlantic *Gymnura* and other batoid species. The effects of trawl fishing and bycatch practices on the physiology of the Gymnuridae should also be a priority, given the high prevalence of these animals in trawl catches. In a recent study examining sub-lethal effects of trawl capture stress on the reproductive capacity of Fiddler Ray (*Trygonorrhina dumerilii*), neonates from stress-exposed mothers were significantly smaller and less fit than neonates from control mothers, highlighting the potential impacts of trawl fishing on the reproductive success and recruitment of other viviparous batoids (Guida et al. 2017). While post-release mortality of U.S. *Gymnura* sp. is assumed to be low, investigations into the physiological effects of common capture and release practices on Butterfly Rays have not been performed. To adequately assess the present status of these populations, species-specific knowledge of the impacts of bycatch fishing practices on the health and survival of gymnurids is vital.

LITERATURE CITED

Alkusairy, H., Ali, M., Saad, A., Reynaud, C. & Capapé, C. (2014). Maturity, reproductive cycle, and fecundity of spiny butterfly ray, *Gymnura altavela* (Elasmobranchii: Rajiformes: Gymnuridae), from the coast of Syria (eastern Mediterranean). Acta Ichthyol Piscat **44,** 229–240.

Bigelow, H. B. & Schroeder, W.C. (1953). Fishes of the Western North Atlantic. Sawfishes, Guitarfishes, Skates, Rays, and Chimaeroids, No. 1, Part 2. Yale University Press. Memoirs Sears Foundation for Marine Research, Yale Univ., New Haven, CT.

Guida, L., Awruch, C., Walker, T.I. & Reina, R.D. (2017). Prenatal stress from trawl capture affects mothers and neonates: a case study using the southern fiddler ray (*Trygonorrhina dumerilii*). Sci. Rep. 7, 46300; doi: 10.1038/srep46300 (2017).

Fisher, R.A., Call, G.C. & Grubbs, R.D. (2013). Age, growth, and reproductive biology of cownose rays (*Rhinoptera bonasus*) in Chesapeake Bay. Mar Coast Fish Dynam Manag Ecosys Sci 5(1):225–234.

Frisk, M.G. (2010). Life history strategies of batoids. In: Carrier, J.C., Musick, J.A., Heithaus, M.R. (eds) Sharks and Their Relatives II: Biodiversity, Adaptive Physiology, and Conservation. CRC Press, Boca Raton, pp 283–316.

Jacobsen, I.P., Johnson, J.W. & Bennett, M.B. (2009). Diet and reproduction in the Australian butterfly ray *Gymnura australis* from northern and north-eastern Australia. J Fish Biol 75:2475–2489.

Naylor, G.J.P., Caira, J.N., Jensen, K., Rosana, K., White, W. & Last, P. (2012). A DNA sequence based approach to the identification of shark and ray species and its implications for global elasmobranch diversity and parasitology. *Bulletin of the American Museum of Natural History*, 367, 263 pp.

Raje, S. G. (2003). Some aspects of biology of four species of rays off Mumbai water. *Indian Journal of Fisheries* **50**, 89–96.

Vooren, C.M., Piercy, A.N., Snelson Jr., F.F., Grubbs, R.D., Notarbartolo di Sciara, G. & Serena, S. (2007). *Gymnura altavela*. The IUCN Red List of Threatened Species 2007: e.T63153A12624290 http://dx.doi.org/10.2305/IUCN.UK.2007.RLTS.T63153A12624290.en Downloaded on 07 July 2017.

White, W. T. & Dharmadi (2007). Species and size compositions and reproductive biology of rays (Chondrichthyes, Batoidea) caught in target and non-target fisheries in eastern Indonesia. *Journal of Fish Biology* **70**, 1809–1837.

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