

Evolution and Phylogeny of Gonad Morphology in Bony Fishes¹

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SYNOPSIS. Gonad morphology at the gross anatomical or histological levels has long been studied by fisheries biologists to identify annual reproductive cycles and length of breeding season, among other goals. Comparative surveys across vertebrate taxa have not been detailed enough, however, to describe fully the differences and similarities among gonads of bony fishes and other vertebrates, and to use gonad morphology in phylogenetic systematic analyses. An emerging constant among vertebrates is the presence of a germinal epithelium composed of somatic and germ cells in both males and females. In females, the germinal epithelium lines the ovarian lamellae. In males, arrangement of the germinal epithelium into compartments varies among osteichthyans: basal taxa have an anastomosing tubular testis, whereas derived taxa have a lobular testis. The lobular testis is proposed as a synapomorphy of the Neoteleostei. The annual reproductive cycle is hypothesized to be the source of morphological variation among testis types. Elongation of germinal compartments during early maturation may result in a transition from anastomosing tubular to lobular testes. In all male atherinomorphs surveyed, spermatogonia are restricted to the distal termini of lobules rather than being distributed along the lobule; there is an epithelioid arrangement of Sertoli and germ cells rather than a germinal epithelium. Arrest of the maturation-regression phases is hypothesized to lead to formation of the atherinomorph testis. Atherinomorphs also have a distinctive egg with fluid, rather than granular, yolk. Variation among germinal epithelia is interpreted in a developing phylogenetic framework to understand evolution of gonad morphology and to propose gonad characters for phylogenetic analyses.

INTRODUCTION

Gonad morphology at the gross anatomical or histological levels has long been studied by fisheries biologists to identify annual reproductive cycles, length of breeding season, onset of reproductive maturity, spawning rhythms, fecundity and various other aspects of reproductive biology that can be applied to fisheries questions and concerns. Of necessity, these studies have focused on a restricted set of commercial or recreational fishing species, such as common snook, *Centropomus undecimalis* (e.g., Grier and Taylor, 1998; Taylor *et al.*, 1998; Neidig *et al.*, 2000), redbfish or red drum, *Sciaenops ocellatus* (e.g., Murphy and Taylor, 1990), bonefish, *Albula vulpes* (e.g., Crabtree *et al.*, 1997), and trout, *Oncorhynchus* species (Billard, 1987), among others. A second field of investigation is the reproduction of marine fishes, many of which have long been known to switch sex and are hermaphroditic (e.g., Warner and Robertson, 1978; Hastings, 1981; Cole, 1988, 1990). A third, well-studied area of fish reproduction is the modes of viviparity in the atherinomorph orders Cyprinodontiformes (*viz.*, Parenti, 1981), including the poeciliids (e.g., Rosen and Bailey, 1963; Hoar, 1969), the four-eyed fishes, genus *Anableps* (e.g., Turner, 1950), and the Mexican goodeids (e.g., Miller and Fitzsimons, 1971), and Belontiiformes, including the viviparous halfbeaks (e.g., Downing and Burns, 1995; Meisner and Burns, 1997;

Meisner, 2001). Despite a few efforts aimed at using reproductive characters in comprehensive classifications of bony fishes (e.g., Breder and Rosen, 1966), these areas of research remain relatively independent. Comparative surveys across vertebrate taxa have not been broad or detailed enough to describe fully the differences and similarities among gonads of bony fishes and other vertebrates, and to use gonad morphology routinely in phylogenetic systematic analyses. These are our aims.

An emerging constant among vertebrates is the presence of a germinal epithelium (Grier, 2000, 2002; Grier and Lo Nostro, 2000). Almost all osteichthyans have a germinal epithelium composed of somatic and germ cells in male and female gonads. We classify the atherinomorph testis as epithelioid based on refinement of definitions of an epithelium as applied to gonad morphology. In teleosts, the germinal epithelium is active throughout the life of the organism and is correlated with indeterminate reproduction of females. Among the Perciformes, five reproductive classes have been described in males of common snook, *Centropomus undecimalis* (see Taylor *et al.*, 1998), spotted sea trout, *Cynoscion nebulosus* (see Brown-Peterson, 2003), cobia, *Rachycentron canadum* (see Brown-Peterson *et al.*, 2002), and the freshwater goby, *Padogobius bonelli* (as *Padogobius martensi*, see Cinquetti and Dramis, 2003):—regressed, early maturation, mid maturation, late maturation, and regression—based on the alternation of the germinal epithelium between continuous and discontinuous types and the stages of germ cells present (Grier and Taylor, 1998; Taylor *et al.*, 1998; Grier, 2002). These changes in the germinal epithelium have also been used to describe annual

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TABLE 1. *Survey of testis types of osteichthyans.**

Species	Testis Type	Reference and/or Material
CLASS SARCOPTERYGII		
Order Coelacanthiformes		
<i>Latimeria chalumnae</i>	anastomosing tubular	Millot <i>et al.</i> , 1978
CLASS ACTINOPTERYGII		
SUBCLASS CHONDROSTEI		
Order Acipenseriformes		
Polyodontidae		
<i>Polyodon spathula</i>	anastomosing tubular	USNM/FMRI
SUBCLASS NEOPTERYGII		
Order Lepisosteiformes		
Lepisosteidae		
<i>Lepisosteus platyrhinchus</i>	anastomosing tubular	Grier, 1993
DIVISION TELEOSTEI		
Order Elopiformes		
Elopidae		
<i>Megalops atlanticus</i>	anastomosing tubular	USNM/FMRI
CLUPEOCEPHALA		
Order Clupeiformes		
Clupeidae		
<i>Dorosoma petenense</i>	anastomosing tubular	Grier, 1993
<i>Opisthonema oglinum</i>	anastomosing tubular	Grier, 1993
Ostariophyi		
Order Cypriniformes		
Cyprinidae		
<i>Abbottina rivularis</i>	anastomosing tubular	USNM 336887
<i>Barbus kahajanii</i>	anastomosing tubular	Grier <i>et al.</i> , 1980
<i>Danio rerio</i>	anastomosing tubular	USNM/FMRI; Maack and Segner, 2003
<i>Notemigonus crysoleucas</i>	anastomosing tubular	Grier <i>et al.</i> , 1980
<i>Notropis hypselopterus</i>	anastomosing tubular	Grier <i>et al.</i> , 1980
Order Characiformes		
Characidae		
<i>Gymnocorymbus ternetzi</i>	anastomosing tubular	Grier <i>et al.</i> , 1980
Order Siluriformes		
Pimelodidae		
<i>Conorhynchus conirostris</i>	anastomosing tubular	Lopes <i>et al.</i> , 2004
Ictaluridae		
<i>Ictalurus natalis</i>	anastomosing tubular	Grier, 1993
SUBDIVISION EUTELEOSTEI		
Protacanthopterygii		
Order Salmoniformes		
Salmonidae		
<i>Oncorhynchus mykiss</i>	anastomosing tubular	USNM/FMRI
<i>Oncorhynchus kisutch</i>	anastomosing tubular	Grier <i>et al.</i> , 1980
NEOGNATHI		
Esociformes		
<i>Esox lucius</i>	anastomosing tubular	Grier <i>et al.</i> , 1980; Grier, 1993
<i>Esox niger</i>	anastomosing tubular	Grier <i>et al.</i> , 1980; Grier, 1993
NEOTELEOSTEI		
Paracanthopterygii		
Order Percopsiformes		
Amblyopsidae		
<i>Amblyopsis spelaea</i>	unrestricted lobular	USNM 127055
Percopsidae		
<i>Percopsis omiscomaycus</i>	unrestricted lobular	USNM 308217
Order Ophidiiformes		
Bythitidae		
<i>Dinematichthys</i> sp.	unrestricted lobular	USNM 338466

TABLE 1. (Continued)

Species	Testis Type	Reference and/or Material
Order Lophiiformes		
Lophiidae		
<i>Lophiodes mutilus</i>	unrestricted lobular	USNM 322221
Order Polymixiiformes		
Polymixiidae		
<i>Polymixia lowei</i>	unrestricted lobular	USNM 157839
SERIES ATHERINOMORPHA		
Order Atheriniformes		
Atherinidae		
<i>Labidesthes sicculus</i>	restricted lobular	Grier <i>et al.</i> , 1980, 1990; USNM 108573
<i>Leuresthes sardina</i>	restricted lobular	USNM 177811
<i>Menidia beryllina</i>	restricted lobular	Grier <i>et al.</i> , 1980
Melanotaeniidae		
<i>Melanotaenia nigrans</i>	restricted lobular	Grier <i>et al.</i> , 1980
Phallostethidae		
<i>Gulaphallus bikolanus</i>	restricted lobular	Grier and Parenti, 1994
<i>Gulaphallus mirabilis</i>	restricted lobular	Grier <i>et al.</i> , 1980; Grier and Parenti, 1994
<i>Neostethus bicornis</i>	restricted lobular	Grier and Parenti, 1994
<i>Neostethus borneensis</i>	restricted lobular	Grier and Parenti, 1994
<i>Neostethus lankesteri</i>	restricted lobular	Grier and Parenti, 1994
<i>Phenacostethus smithi</i>	restricted lobular	Munro and Mok, 1990; Grier and Parenti, 1994
<i>Phenacostethus posthon</i>	restricted lobular	Grier and Parenti, 1994
Order Cyprinodontiformes		
Aplocheilidae		
<i>Aphyosemion gardneri</i>	restricted lobular	USNM 339706
Rivulidae		
<i>Pterolebias hoignei</i>	restricted lobular	USNM 245947
Profundulidae		
<i>Profundulus guatemalensis</i>	restricted lobular	USNM 134600
Fundulidae		
<i>Adinia xenica</i>	restricted lobular	Grier <i>et al.</i> , 1980
<i>Fundulus chrysotus</i>	restricted lobular	Grier <i>et al.</i> , 1980
<i>Fundulus grandis</i>	restricted lobular	Grier <i>et al.</i> , 1980; USNM/FMRI
<i>Fundulus seminolis</i>	restricted lobular	Grier <i>et al.</i> , 1980
<i>Lucania goodei</i>	restricted lobular	Grier <i>et al.</i> , 1980
Goodeidae		
<i>Ameca splendens</i>	restricted lobular	Grier <i>et al.</i> , 1980
<i>Ataeniobius toweri</i>	restricted lobular	Grier <i>et al.</i> , 1980
<i>Characodon lateralis</i>	restricted lobular	Grier <i>et al.</i> , 1980
<i>Xenotoca eiseni</i>	restricted lobular	Grier <i>et al.</i> , 1980; USNM 374494
Anablepidae		
<i>Anableps anableps</i>	restricted lobular	Grier <i>et al.</i> , 1980
<i>Anableps dowi</i>	restricted lobular	Grier <i>et al.</i> , 1980
<i>Jenynsia lineata</i>	restricted lobular	Grier <i>et al.</i> , 1980
<i>Jenynsia multidentata</i>	restricted lobular	Martínez and Monasterio de Gonzo, 2002
Poeciliidae		
<i>Cnesterodon decemmaculatus</i>	restricted lobular	USNM 360480
<i>Gambusia affinis</i>	restricted lobular	Grier <i>et al.</i> , 1980
<i>Heterandria formosa</i>	restricted lobular	Grier <i>et al.</i> , 1980
<i>Poecilia latipinna</i>	restricted lobular	Grier <i>et al.</i> , 1980
<i>Poecilia reticulata</i>	restricted lobular	Grier <i>et al.</i> , 1980
<i>Poeciliopsis gracilis</i>	restricted lobular	Grier <i>et al.</i> , 1980
<i>Tomeurus gracilis</i>	restricted lobular	Grier <i>et al.</i> , 1980; USNM 225463
<i>Xiphophorus helleri</i>	restricted lobular	Grier <i>et al.</i> , 1980
<i>Xiphophorus maculatus</i>	restricted lobular	Grier <i>et al.</i> , 1980
Cyprinodontidae		
<i>Cyprinodon variegatus</i>	restricted lobular	Grier <i>et al.</i> , 1980
<i>Jordanella floridae</i>	restricted lobular	Grier <i>et al.</i> , 1980

TABLE 1. (Continued)

Species	Testis Type	Reference and/or Material
Order Beloniformes		
Adrianichthyidae		
<i>Horaichthys setnai</i>	restricted lobular	Grier, 1984
<i>Oryzias latipes</i>	restricted lobular	Grier, 1976
<i>Oryzias matanensis</i>	restricted lobular	USNM 340428
Exocoetidae		
<i>Cypselurus heterurus</i>	restricted lobular	USNM 294785
<i>Hirundichthys speculiger</i>	restricted lobular	USNM 299274
<i>Oxyporhamphus micropterus</i>	restricted lobular	USNM 216327
<i>Prognichthys gibbifrons</i>	restricted lobular	USNM 185882, 185883
Hemiramphidae		
<i>Dermogenys bispinna</i>	restricted lobular	Downing and Burns, 1995
<i>Dermogenys burmanica</i>	restricted lobular	Downing and Burns, 1995; Meisner, 2001
<i>Dermogenys orientalis</i>	restricted lobular	Downing and Burns, 1995
<i>Dermogenys pusilla</i>	restricted lobular	Grier <i>et al.</i> , 1980; Downing and Burns, 1995
<i>Dermogenys siamensis</i>	restricted lobular	Downing and Burns 1995; Meisner, 2001
<i>Euleptorhamphus velox</i>	restricted lobular	Grier <i>et al.</i> , 1980
<i>Hemiramphus brasiliensis</i>	restricted lobular	Grier <i>et al.</i> , 1980; USNM/FMRI
<i>Hemiramphodon chryopunctatus</i>	restricted lobular	Downing and Burns, 1995
<i>Hemiramphodon kapuasensis</i>	restricted lobular	Downing and Burns, 1995
<i>Hemiramphodon kuekenthali</i>	restricted lobular	Downing and Burns, 1995
<i>Hemiramphodon phaisoma</i>	restricted lobular	Downing and Burns, 1995
<i>Hemiramphodon pogonognathus</i>	restricted lobular	Downing and Burns, 1995
<i>Hemiramphodon tengah</i>	restricted lobular	Downing and Burns, 1995
<i>Hyporhamphus quoyi</i>	restricted lobular	Grier <i>et al.</i> , 1980
<i>Hyporhamphus regularis</i>	restricted lobular	Grier <i>et al.</i> , 1980
<i>Nomorhamphus brembachi</i>	restricted lobular	Downing and Burns, 1995; Meisner, 2001
<i>Nomorhamphus celebensis</i>	restricted lobular	Downing and Burns, 1995
<i>Nomorhamphus ebrardtii</i>	restricted lobular	Downing and Burns, 1995; Meisner, 2001
<i>Nomorhamphus liemi</i>	restricted lobular	Downing and Burns, 1995
<i>Nomorhamphus rossi</i>	restricted lobular	Downing and Burns, 1995; Meisner, 2001
<i>Nomorhamphus towoetii</i>	restricted lobular	Downing and Burns, 1995
<i>Nomorhamphus vivipara</i>	restricted lobular	Downing and Burns, 1995; Meisner, 2001
<i>Nomorhamphus weberi</i>	restricted lobular	Downing and Burns, 1995; Meisner, 2001
<i>Zenarchopterus buffonis</i>	restricted lobular	Grier and Collette, 1987
<i>Zenarchopterus caudovittatus</i>	restricted lobular	Grier and Collette, 1987
<i>Zenarchopterus dispar</i>	restricted lobular	Grier and Collette, 1987
<i>Zenarchopterus dunckeri</i>	restricted lobular	Grier and Collette, 1987
<i>Zenarchopterus ectuntio</i>	restricted lobular	Grier and Collette, 1987
<i>Zenarchopterus gilli</i>	restricted lobular	Grier and Collette, 1987
<i>Zenarchopterus kampeni</i>	restricted lobular	Grier and Collette, 1987
<i>Zenarchopterus novaeguineae</i>	restricted lobular	Grier and Collette, 1987
<i>Zenarchopterus ornithocephala</i>	restricted lobular	Grier and Collette, 1987
<i>Zenarchopterus rasori</i>	restricted lobular	Grier and Collette, 1987
<i>Zenarchopterus robertsi</i>	restricted lobular	Grier and Collette, 1987
SERIES MUGILOMORPHA		
Mugilidae		
<i>Agnostomus monticola</i>	unrestricted lobular	USNM 318360
<i>Mugil cephalus</i>	unrestricted lobular	USNM 101188; 111387; Grier <i>et al.</i> , 1980
SERIES PERCOMORPHA		
Order Synbranchiformes		
Synbranchidae		
<i>Synbranchus marmoratus</i>	unrestricted lobular	LoNostro <i>et al.</i> , 2003
Mastacembelidae		
<i>Mastacembelus armatus</i>	unrestricted lobular	USNM 319481
Order Gasterosteiformes		
Gasterosteidae		
<i>Pungitius sinensis</i>	unrestricted lobular	USNM 336886
Order Perciformes		
Elassomatoidei		
Elassomatidae		
<i>Elassoma evergladei</i>	unrestricted lobular	USNM 357366

TABLE 1. (Continued)

Species	Testis Type	Reference and/or Material
Percoidei		
Centrarchidae		
<i>Enneacanthus gloriosus</i>	unrestricted lobular	Grier <i>et al.</i> , 1980
<i>Lepomis macrochirus</i>	unrestricted lobular	Grier <i>et al.</i> , 1980
<i>Micropterus salmoides</i>	unrestricted lobular	Grier <i>et al.</i> , 1980
<i>Pomoxis nigromaculatus</i>	unrestricted lobular	Grier <i>et al.</i> , 1980
Centropomidae		
<i>Centropomus undecimalis</i>	unrestricted lobular	Taylor <i>et al.</i> , 1998
Percidae		
<i>Perca flavescens</i>	unrestricted lobular	Grier <i>et al.</i> , 1980
Rachycentridae		
<i>Rachycentron canadum</i>	unrestricted lobular	Brown-Peterson <i>et al.</i> , 2002
Gerreidae		
<i>Diapterus plumieri</i>	unrestricted lobular	Grier <i>et al.</i> , 1980
Serranidae		
<i>Centropristis striata</i>	unrestricted lobular	USNM/FMRI
Sparidae		
<i>Archosargus probatocephalus</i>	unrestricted lobular	Grier <i>et al.</i> , 1980
Sciaenidae		
<i>Cynoscion nebulosus</i>	unrestricted lobular	Brown-Peterson, 2003
<i>Sciaenops ocellatus</i>	unrestricted lobular	Grier, 1993
Labroidei		
Labridae		
<i>Thalassoma bifasciatum</i>	unrestricted lobular	Koulish <i>et al.</i> , 2002
Cichlidae		
<i>Cichlasoma nigrofasciatum</i>	unrestricted lobular	Grier <i>et al.</i> , 1980
<i>Labeotropheus trewavasae</i>	unrestricted lobular	Grier <i>et al.</i> , 1980
<i>Oreochromis</i> sp.	unrestricted lobular	Grier, 1993
<i>Pterophyllum scalare</i>	unrestricted lobular	Grier <i>et al.</i> , 1980
<i>Sarotherodon aurea</i>	unrestricted lobular	Grier <i>et al.</i> , 1980
Blennioidei		
Blenniidae		
<i>Ophioblennius steindachneri</i>	unrestricted lobular	USNM 292427
Gobioidei		
Odontobutidae		
<i>Micropercops swinhonis</i>	unrestricted lobular	USNM 336883
Microdesmidae		
<i>Microdesmus bahianus</i>	unrestricted lobular	Thacker and Grier, 2004
<i>Microdesmus dorsipunctatus</i>	unrestricted lobular	Thacker and Grier, 2004
Gobiidae		
<i>Bathygobius lineatus</i>	unrestricted lobular	Thacker and Grier, 2004
<i>Neogobius fluviatilis</i>	unrestricted lobular	Thacker and Grier, 2004
<i>Padogobius bonelli</i>	unrestricted lobular	Cinquetti and Dramis, 2003
<i>Pandaka pygmaea</i>	unrestricted lobular	Thacker and Grier, 2004
<i>Tridentiger bifasciatus</i>	unrestricted lobular	Thacker and Grier, 2004
Schindleriidae		
<i>Schindleria praematura</i>	restricted lobular	Thacker and Grier, 2004

* Classification follows Nelson (1994), Johnson and Patterson (1996), and Parenti (2004), in part. See text for further explanation.

male reproductive classes in the synbranchiform swamp eel, *Synbranchus marmoratus* (see Lo Nostro *et al.*, 2003). Further, arrangement of the male germinal epithelium into compartments is a fixed characteristic among taxa that may be used to define testis types (Grier, 1993).

One diagnostic character of the atherinomorph fishes was described by Rosen and Parenti (1981, p. 11) as "... spermatogonia ... restricted to the distal end of the tubule immediately beneath the tunica albuginea whereas other groups of teleosts have the spermatogonia distributed along the length of the tubule." The

previous year, Grier *et al.*, (1980) identified this distinctive testis type and reported it in 31 atherinomorph species representing each of the three currently recognized orders (*viz.*, Parenti, 2004). The more taxonomically widespread condition was reported in 19 teleost species, including *Esox* and *Oncorhynchus* species, as well as an array of ostariophysans and percormorphs (Table 1). Description and definition of testis types in osteichthyans was reconsidered by Grier (1993) who concluded that primitive osteichthyans have an anastomosing tubular testis, whereas derived teleosts, including atherinomorphs, have a lobular testis, and that the lobular testis could be divided into two types based on distribution and arrangement of spermatogonia. Germinal compartments that extend to the periphery of the testis and terminate blindly are termed lobules, not tubules (Figs. 1A–D; 2A–D; see Grier, 1993, and Discussion, below). Hence, the atherinomorph testis has a restricted distribution of spermatogonia at the distal ends of lobules (Fig. 1A,C,D), in contrast to the “perciform testis type,” so-called because of its initial description in fishes at one time classified in the order Perciformes, such as the striped mullet, *Mugil cephalus* (Fig. 1B), in which spermatogonia are distributed along the lengths of testis lobules. Surveys of gonad morphology during the past two decades have confirmed the presence of the unique, restricted testis type in atherinomorphs (*viz.*, Grier and Collette, 1987; Grier and Parenti, 1994; Downing and Burns, 1995; Figs. 1, 2; Table 1). There have been no proposals, however, regarding the hierarchical level at which we can recognize the lobular testis as a synapomorphy.

Our collaboration began as an attempt to clarify both the definition of testis types and their distribution among bony fish taxa. It has expanded necessarily to include ovarian and egg characters as they also vary phylogenetically (Figs. 3, 4). Variation among germinal epithelia is interpreted in a developing phylogenetic framework to understand the evolution of gonad morphology and also to begin to propose gonad characters that may be used in phylogenetic analyses.

MATERIALS AND METHODS

Gonad material was obtained from either freshly fixed specimens or from specimens maintained in the fish collection of the National Museum of Natural History (USNM), Smithsonian Institution. The museum material is identified by the prefix USNM (United States National Museum), followed by a catalogue number. Material that was collected and processed at the Florida Marine Research Institute (now the Fish and Wildlife Research Institute) is identified by the abbreviation USNM/FMRI. Voucher material will be deposited in the permanent collections of the USNM. Paddlefish, *Polyodon spathula*, gonads were obtained from Kentucky State University. Rainbow trout, *Oncorhynchus mykiss*, gonads were obtained from the El Zarco trout hatchery outside of Mexico City, Mexico.

Testis type was recorded in 136 osteichthyan species from our own observations, the literature or both (Table 1). Egg type was observed in a more limited set of taxa and is cited in the text.

The museum material is likely to have been fixed in formalin, a common fixative starting in the late 1800s. It is currently stored in 75% ethanol. Gonads from fresh material were fixed in Bouin's solution or buffered 10% formalin. Formalin-fixed, alcohol-preserved whole fish specimens stored in museum collections for decades proved as useful and reliable for examination of histological structure of gonads as recently fixed material. Museum collections are the only source of gonads of certain taxa. When museum specimens did not prove to be of high histological quality, our opinion was that the initial fixation was at fault, not the prolonged storage in ethanol.

Whole or sectioned gonads were embedded in plastic (glycolmethacrylate [Polysciences]) or paraffin. Tissue sections were cut at 6–8 μm (paraffin) or 3.5 and 4 μm (plastic). Paraffin sections were stained with hematoxylin and eosin; plastic sections were stained with thionin or metanil yellow-periodic acid/Schiff's (PAS) hematoxylin (Quintero-Hunter *et al.*, 1991).

RESULTS

Testes

Anastomosing tubular testes are found throughout primitive teleost taxa, ranging from the tarpon, *Megalops atlanticus* (Fig. 2E), the cypriniform, *Abbottina rivularis* (Fig. 2C), to the rainbow trout, *Oncorhynchus mykiss* and the pikes and pickerels, genus *Esox* (see Table 1 for references and material). In some histological preparations of anastomosing tubular testes, the germinal compartments may appear somewhat lobular, as in Figure 2E, probably owing to the plane of section through a three-dimensional tissue. Our descriptions are based upon two-dimensional histological representations of the three-dimensional germinal compartments. A tubular testis, which appears to be anastomosing, characterizes the primitive sarcopterygian, the coelacanth, *Latimeria chalumnae*, and the primitive non-teleost actinopterygians, the paddlefish, *Polyodon spathula*, and the gar, *Lepisosteus platyrhinchus* (Table 1).

Lobular testes characterize all fishes of the Neoteleostei that we have surveyed or for which we found citations (Figs. 1, 2A,B,D; Table 1). Lobular testes may be further divided into restricted and unrestricted types (Grier *et al.*, 1980; Grier, 1993). In all male atherinomorph fishes surveyed to date, 79 species representing all three orders, including taxa with a range of reproductive modes, spermatogonia are restricted to the distal termini of lobules rather than being distributed along the lobule (Fig. 1A,C,D; Table 1). Furthermore, there is an epithelioid arrangement of Sertoli and germ cells; that is, the germ cells and Sertoli cells

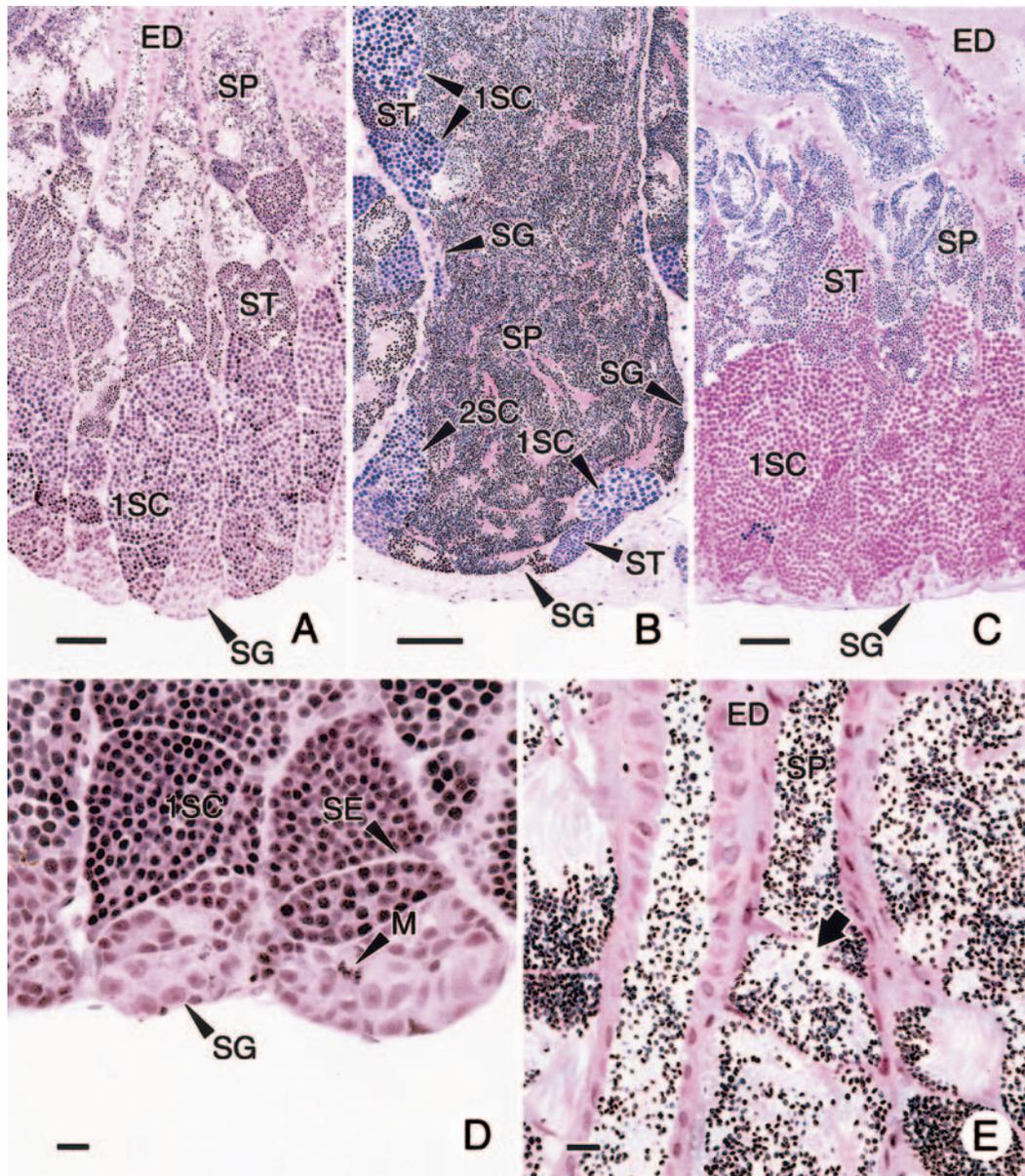


FIG. 1. A. Cross section of the testis from the Gulf killifish, *Fundulus grandis*. The lobules terminate at the periphery of the testis, where spermatogonia (SG) are located. Proceeding proximally, meiotic germ cells are arranged almost in rows between juxtaposed lobules, and primary spermatocytes (1SC), spermatids (ST), and sperm (SP) in spermatocysts or within the lumina of efferent ducts (ED) are observed. Bar = 50 μm . B. A lobule from the striped mullet, *Mugil cephalus*, collected in late October, approximately one month before spawning condition. Sperm (SP) accumulate within the lobule lumen. Spermatogonia (SG) are observed both at the distal terminus of the lobule and also along the lateral walls as are spermatocysts containing primary spermatocytes (1SC), secondary spermatocytes (2SC), and spermatids (ST). The germinal epithelium has become discontinuous as extensive regions of the lobule lack spermatocysts. Bar = 50 μm . C. Testis lobules from the ballyhoo, *Hemiramphus brasiliensis*, have spermatogonia (SG) restricted to their distal termini. Later stage developing sperm within spermatocysts, primary spermatocytes (1SC) and spermatids (ST) are located progressively closer to the efferent ducts (ED) which are filled with sperm (SP). Bar = 50 μm . D. The distal termini of lobules from the testis of *Fundulus grandis*. Spermatogonia (SG), some dividing and in metaphase (M), are restricted to the lobule distal termini. The borders of spermatocysts with primary spermatocytes (1SC) are delineated by lightly-staining Sertoli cells (SE). Bar = 10 μm . E. The efferent duct (ED) cells in the testis of *Fundulus grandis* contain eosinophilic secretory material (bright pink). At the arrow, spermiogenesis, or release of sperm into the efferent ducts is occurring as Sertoli cell processes separate, the lumen of the spermatocyst then becomes continuous with that of the efferent duct. Bar = 10 μm .

within the lobules do not border directly onto a lumen. By definition, epithelia border a body surface, lumen, or tube (*viz.*, Grier, 2000; Grier and Lo Nostro, 2000). In atherinomorphs, the Sertoli cells extend processes

across the widths of the lobules; spermatocysts, therefore, extend across the lobules, and there is no lumen within the lobule (Fig. 1A,C,D). At spermiation, sperm are voided from the spermatocyst the lumen of which

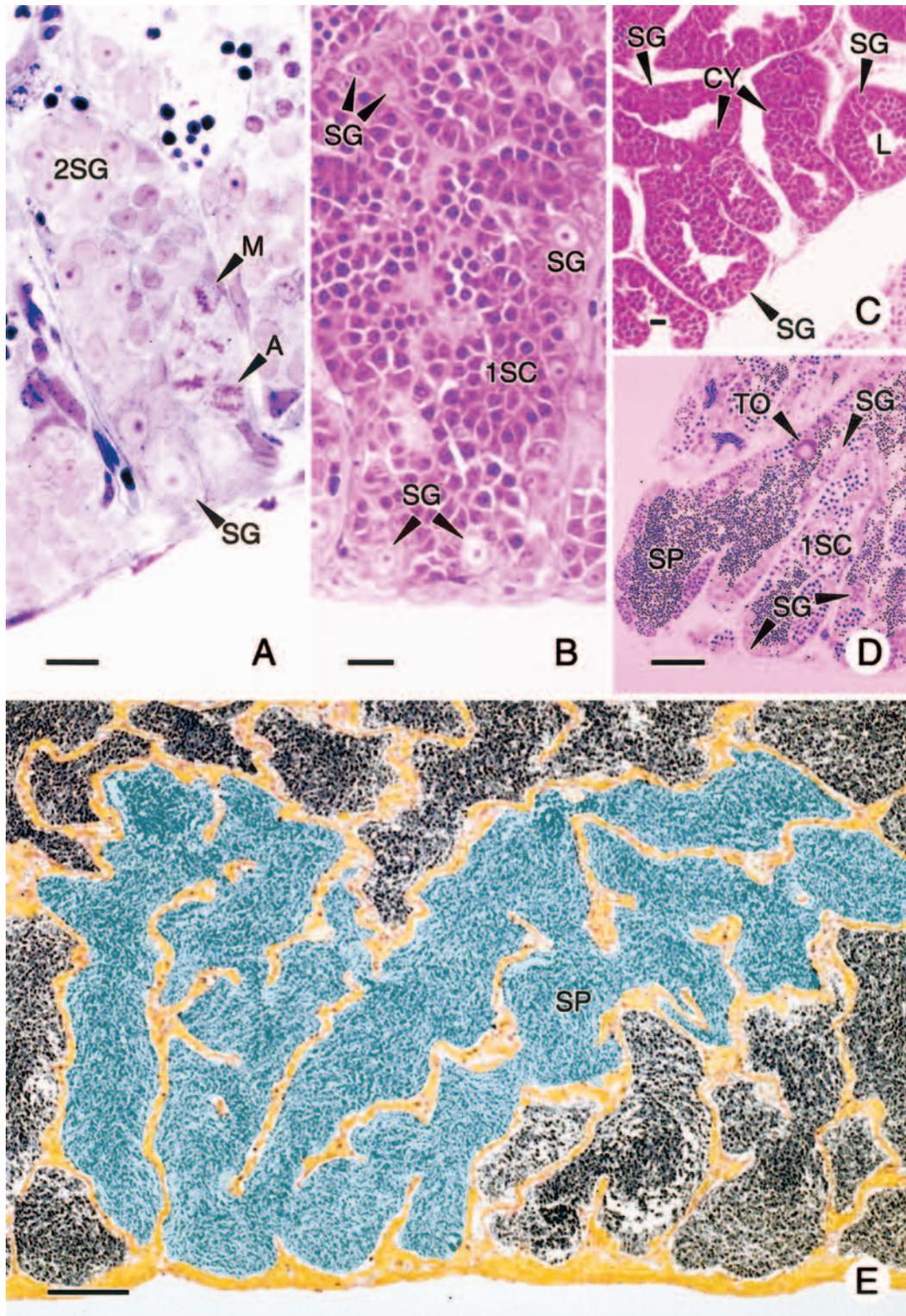


FIG. 2. A. A lobule from the Everglades pygmy sunfish, *Elassoma evergladei*, illustrating primary spermatogonia (SG) with different-sized nuclei. Some spermatogonia are in metaphase (M) or anaphase (A) along the wall of the lobule. Secondary spermatogonia (2SG) are within spermatocysts. Bar = 10 μ m. B. A testicular lobule from the trout-perch, *Percopsis omiscomaycus*, illustrating spermatogonia (SG) at the distal terminus of and along the lobule wall. The lobule is occluded, and no lumen is observed, with spermatocysts containing synchronously-developing primary spermatocytes (1SC). Bar = 10 μ m. C. Testicular lobules from the Chinese false gudgeon, *Abbottina rivularis*. Spermatocysts (CY) containing spermatocytes are numerous, and a few scattered spermatogonia (SG) are located at the distal termini and along the

becomes continuous with that of the efferent duct (Fig. 1E).

Ovaries and oocytes

The ovarian germinal epithelium is the origin of follicles in the fish ovary (Grier, 2000), actively producing follicles throughout the annual reproductive cycle in the perciform *Centropomus undecimalis*. Morphology of the germinal epithelium is identical in *C. undecimalis* and the synbranchiform *Synbranchus marmoratus* (see Ravaglia and Maggese, 2003). In the atherinomorph Gulf killifish, *Fundulus grandis* (Fig. 3A), the ovarian germinal epithelium has essentially the same features as the above two species, and separates the ovarian lumen from the stroma. A consistent feature of the ovarian stroma in teleosts is a conspicuous extravascular space of varying size, and sometimes seemingly nonexistent. If they do not dissolve in histological preparation, the fixed "fluids" within the extravascular space stain positively, if only lightly, for glycoproteins as does blood plasma. As capillaries are "leaky," that is, their endothelial cells are not joined by tight junctions, we infer that fluids within the extravascular space are derived from the circulatory system. Furthermore, their demonstration may be dependent upon type of fixative, rate of penetration of fixative, and stain (Grier, unreported).

The germinal epithelium is composed of epithelial cells that become prefollicle cells when associated with oogonia, as in *Fundulus grandis* (Fig. 3A). It is subtended by prethecal cells that are identical to cells in the ovarian stroma (Grier, unreported). Once an oogonium enters meiosis, it becomes an oocyte that progresses through the initial phases of the first meiotic prophase until the diplotene stage when division is arrested, and the other events of oocyte maturation commence: primary oocyte growth (previtellogenesis [Patiño and Sullivan, 2002], Fig. 3A,B), secondary oocyte growth (vitellogenesis [Patiño and Sullivan, 2002], Fig. 3B,C), and final oocyte maturation (Figs. 3D, 4A). During primary growth, the yolk nucleus appears as do cortical alveoli (Fig. 3B).

From the onset of formation of oocyte cytoplasmic yolk, osteichthyans have yolk that is distinctly granular, *i.e.*, during vitellogenesis, the process of yolk formation, protein yolk is formed into spherical globules (Fig. 4B). Atherinomorphs have what we describe as fluid yolk; it is relatively uniform in contrast to that of other osteichthyans (Figs. 3C,D, 4A). The difference between fluid and granular yolk is discerned readily histologically. Granular yolk becomes fluid during the final, pre-ovulatory events of oocyte maturation

(Wallace and Selman, 1981; Selman and Wallace, 1989; Neidig *et al.*, 2000), to which the term "final oocyte maturation" (FOM) has been applied (Jalabert *et al.*, 1977). In atherinomorphs, protein yolk appears globular at the oocyte surface, but these globules continuously fuse (Fig. 3C) to form a yolky mass that stains positively with the periodic acid Schiff reaction for glycoproteins.

The events of final oocyte maturation have not been documented in atherinomorphs, but in *Hemiramphus brasiliensis* (see McBride and Thurman, 2003; Fig. 4A) there is both an increase in oocyte diameter and a reduction of the periodic acid Schiff staining of yolk. In contrast to the fluid yolk of atherinomorphs, yolk is organized into globules in other taxa. In *Mugil cephalus* (Fig. 4B), yolk is distinctively globular and eosinophilic, not staining with periodic acid Schiff. In *Elassoma evergladei* (Fig. 4C), the yolk is periodic acid Schiff-positive, but globular.

DISCUSSION AND CONCLUSIONS

Our survey confirms the initial observation of Grier (1993) that an anastomosing tubular testis characterizes basal osteichthyans, including basal teleosts, whereas a lobular testis characterizes higher teleosts (Table 1). The lobular testis type is proposed as a diagnostic or synapomorphic character of the Neoteleostei, as discussed below. Further, we confirm the results of Grier *et al.* (1980) that the restricted lobular type of testis is diagnostic of atherinomorph fishes.

Testis types in fishes have been defined poorly, based on examination of too few species or indiscriminate application of terms such that "lobule" and "tubule" were used interchangeably. For example, Grier *et al.* (1980) noted that the literature does not "clearly distinguish structural differences between "lobular" and "tubular" testicular types," and that use of either term varied by author. The testes of fishes, both the basal osteichthyans and the neoteleosts, were termed "tubular" (Grier *et al.*, 1980; Grier, 1981) deliberately to avoid confusion with the erroneous concept that some fishes have "lobule" boundary cells as Leydig cell homologs. It was demonstrated subsequently that one teleost species, *Esox lucius*, which was reported to have lobule boundary cells as Leydig cell homologs, had a typical distribution of interstitial, hormone-secreting Leydig cells (Grier *et al.*, 1989). The so-called lobule boundary cells were Sertoli cells within the germinal compartment. Further comparative investigations of testis morphology in teleosts led to a new interpretation: the basal osteichthyans have anastomosing tubular testes in which the germinal compart-

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FIG. 2. (Continued) lobule (L) walls. Bar = 10 μm . D. Testis lobules of the beardfish, *Polymixia lowei*, have spermatogonia (SG) located along their walls and at the distal termini. Spermatocytes (SC) and one testis ovum (TO) are observed. Bar = 50 μm . E. The anastomosing tubular testis of the tarpon, *Megalops atlanticus*, is illustrated. Anastomosing tubules do not terminate at the testis periphery, but rather form continuous, interconnected loops as observed in the lobules shaded in turquoise. Lobules to the right of those shaded appear to terminate at the periphery of the testis owing to plane of section. The lobules in this reproductive fish are filled with stored sperm, and developing sperm in spermatocysts are not observed, a characteristic of reproductive, synchronous spawning fish. Bar = 100 μm .

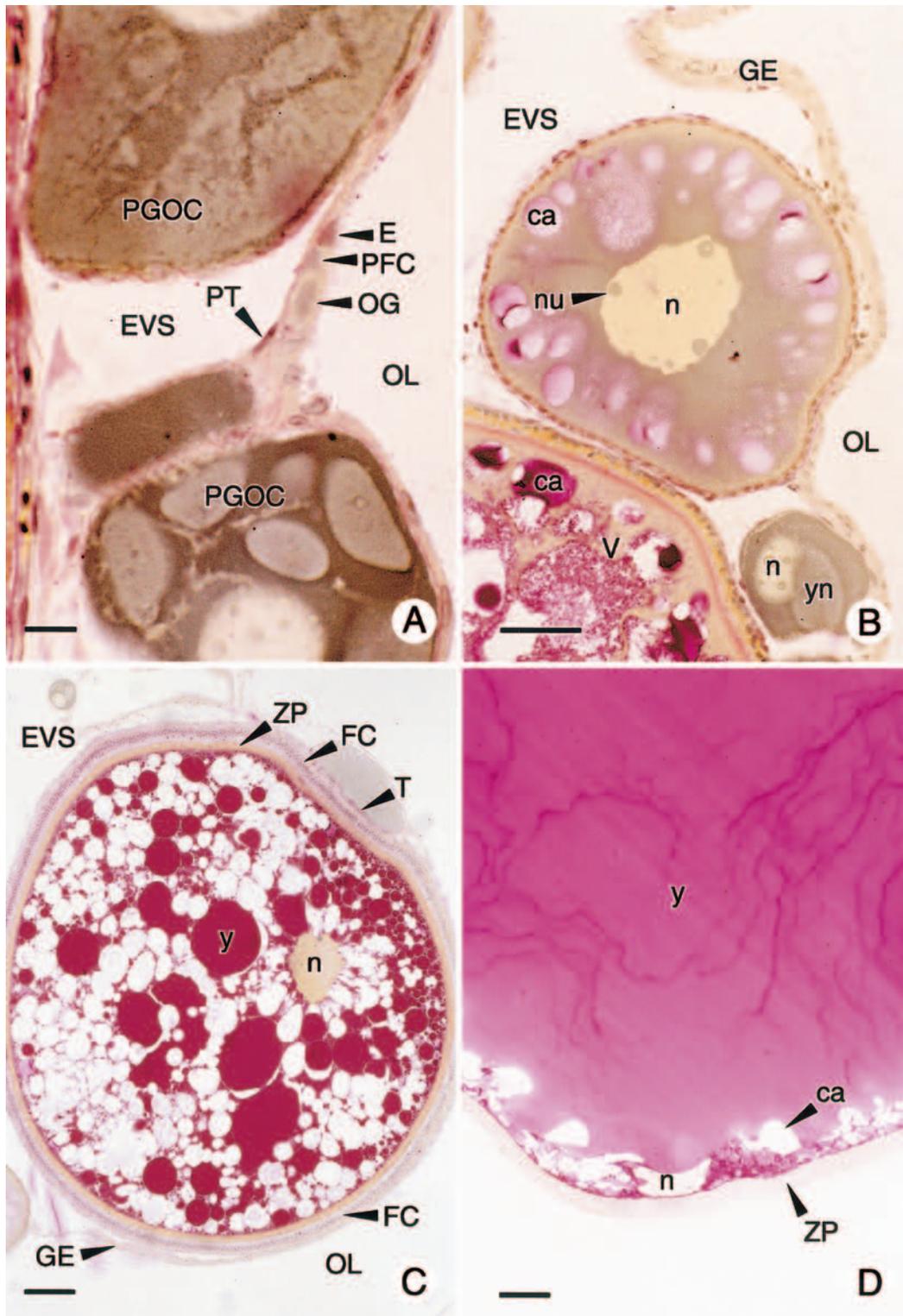


FIG. 3. A. The germinal epithelium of *Fundulus grandis* borders the ovarian lumen (OL). In it are observed an oogonium (OG) associated with a prefollicle cell (PFC), distal to which is an epithelial cell (E). Epithelial cells that are associated with oogonia are prefollicle cells. Beneath the germinal epithelium is a prethecal cell (PT). An extravascular space (EVS) is prominent, and primary growth oocytes (PGOC), with dense-staining cytoplasm, are located beneath the germinal epithelium. Bar = 10 μ m. B. In *Fundulus grandis* the germinal epithelium separates the ovarian lumen (OL) from an extensive extravascular space (EVS) in which a primary growth oocyte, with cortical alveoli (ca), is observed. Its nucleoli (nu) are located around the periphery of the nucleus (n). A smaller primary growth oocyte has a prominent yolk nucleus (yn) within its cytoplasm, and the nucleoli are scattered within the nucleus (n). Cortical alveoli are also observed within a vitellogenic

ments do not terminate at the testis periphery, but form highly branched, anastomosing loops or tubules. The term “tubule” was retained as for mammals in which testicular tubules do not terminate at the testis periphery, but also form loops (*viz.*, Grier, 1993). In neoteleosts, the germinal compartments may form anastomosing networks proximally, but distally they extend to the periphery of the testis and terminate blindly. This character defines the lobular testis, which is either unrestricted (neoteleosts) or restricted (atherinomorphs) with regard to the distribution of spermatogonia within the lobules (Grier, 1993). Furthermore, the testes in Atherinomorpha do not form anastomosing networks, but the lobules may branch as they extend from the efferent ducts to the periphery of the testis. This may be another atherinomorph synapomorphy, but support for more than this simple proposal is lacking. The description of the germinal compartments in atherinomorphs as “lobular” (Grier, 1993) replaces use of the term “tubular” (Grier *et al.*, 1980; Grier, 1981; Nagahama, 1983; Rosen and Parenti, 1981) and emphasizes the distinctive differences in testis structure between basal and higher teleosts as subsequently defined by Grier (1993). The testis of the Everglades pygmy sunfish, *Elassoma evergladei*, a species examined here, is lobular, not anastomosing tubular as described for the banded pygmy sunfish *Elassoma zonatum* by Walsh and Burr (1984). We interpret the testis of *E. zonatum* as unrestricted lobular and suggest that description of the testis as anastomosing tubular was due to the inconsistent way in which these terms have been applied.

The unrestricted lobular testis is found throughout the Neoteleostei (Table 1), including the paracanthopterygian *Percopsis* (Fig. 2B), the beardfish *Polymixia* (Fig. 2D), and all other neoteleosts, except for the atherinomorphs and the diminutive gobioid *Schindleria*, discussed below. Basal, deep-sea neoteleosts in the orders Myctophiformes, Stomiiformes, and Aulopiformes (following the classification of Johnson and Patterson, 1996) have not been surveyed; that they have an unrestricted lobular testis is a prediction open to test.

Atherinomorphs have been proposed by morphologists to be closely related to the percomorphs (Rosen and Parenti, 1981), to mullets (Stiassny, 1993), to a group of taxa in a higher-level teleost category, the Smegmamorpha (Johnson and Patterson, 1993), or with unresolved relationships to the percomorphs and paracanthopterygians (Parenti, 1993). Smegmamorphs

were considered by Johnson and Patterson (1993) to include, in addition to the atherinomorphs, the mullets, Mugilidae, the swamp and spiny eels, Synbranchiformes, the sticklebacks and relatives, Gasterosteiformes, and the pygmy sunfishes, *Elassoma*. We examined representatives of each of the non-atherinomorph smegmamorphs and found all to have an unrestricted lobular testis (Table 1). Similarly, all paracanthopterygians examined have an unrestricted lobular testis (Table 1).

That the restricted lobular testis of atherinomorph fishes is not found in any of the other proposed smegmamorphs does not test smegmamorph monophyly, but does refute a recent molecular hypothesis in which atherinomorph monophyly was challenged (*e.g.*, Chen *et al.*, 2003). Atherinomorph monophyly has not been questioned by morphologists since Gosline (1971), and has been corroborated in a recent, broad-scale molecular analysis (Miya *et al.*, 2003). The list of diagnostic morphological characters for atherinomorphs is extensive and includes characters of the egg (*viz.*, Parenti, 2004) to which we add fluid, rather than granular, yolk. All other so-called smegmamorphs have globular protein yolk within vitellogenic and mature oocytes (Fig. 4B,C). Only during final oocyte maturation, preceding ovulation, does the yolk become fluid—as observed throughout vitellogenesis in atherinomorphs (Fig. 4A). We also note that atherinomorphs have periodic acid Schiff-positive yolk, but the phylogenetic significance of this observation remains to be determined with further surveys.

Ultrastructural investigation has shown that oogonia are scattered in the simple epithelium lining the ovarian cavity or lumen in common snook, *Centropomus undecimalis* (see Grier, 2000) and the swamp eel, *Synbranchus marmoratus* (see Ravaglia and Maggese, 2003). It is a discontinuous germinal epithelium, following definitions of germinal epithelia (continuous and discontinuous) developed for common snook (Grier and Taylor, 1998). Our examination of the ovary of *Fundulus grandis*, using plastic embedded tissue, revealed the presence of oogonia that are scattered within a germinal epithelium, similarly associated with epithelial and prefollicle cells as in the snook and the swamp eel. Contrary to Brummett *et al.* (1982), who indicated that “oocytes appear to be derived from oogonia located immediately beneath the simple epithelium lining the ovarian cavity,” we conclude that the luminal epithelium in the ovary of *Fundulus* is the germinal epithelium. Furthermore, it is established that

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FIG. 3. (Continued) oocyte (V). Bar = 50 μm . C. In vitellogenic oocytes of *Fundulus grandis* the yolk globules (y) fuse, being quite separate and small at the oocyte periphery and larger centrally. Because of staining characteristics, it is difficult to distinguish between fat globules and cortical alveoli which become progressively lighter-staining as oocyte development proceeds. The oocyte is surrounded by a zona pellucida (ZP), often called a chorion. Exterior to this is a follicle cell (FC) layer, and theca (T). The oocyte nucleus (n) is dwarfed by the increasing size of the oocyte. Germinal epithelium (GE). Extravascular space, (EVS). Bar = 100 μm . D. Portion of a mature oocyte from *Fundulus grandis* wherein the cytoplasm is filled with periodic acid Schiff-positive fluid, protein yolk. Cortical alveoli (ca) are restricted to the periphery of the oocyte, as is the nucleus (n), indicating that this oocyte is preovulatory. The oocyte is surrounded by the zona pellucida (zp). Bar = 100 μm .

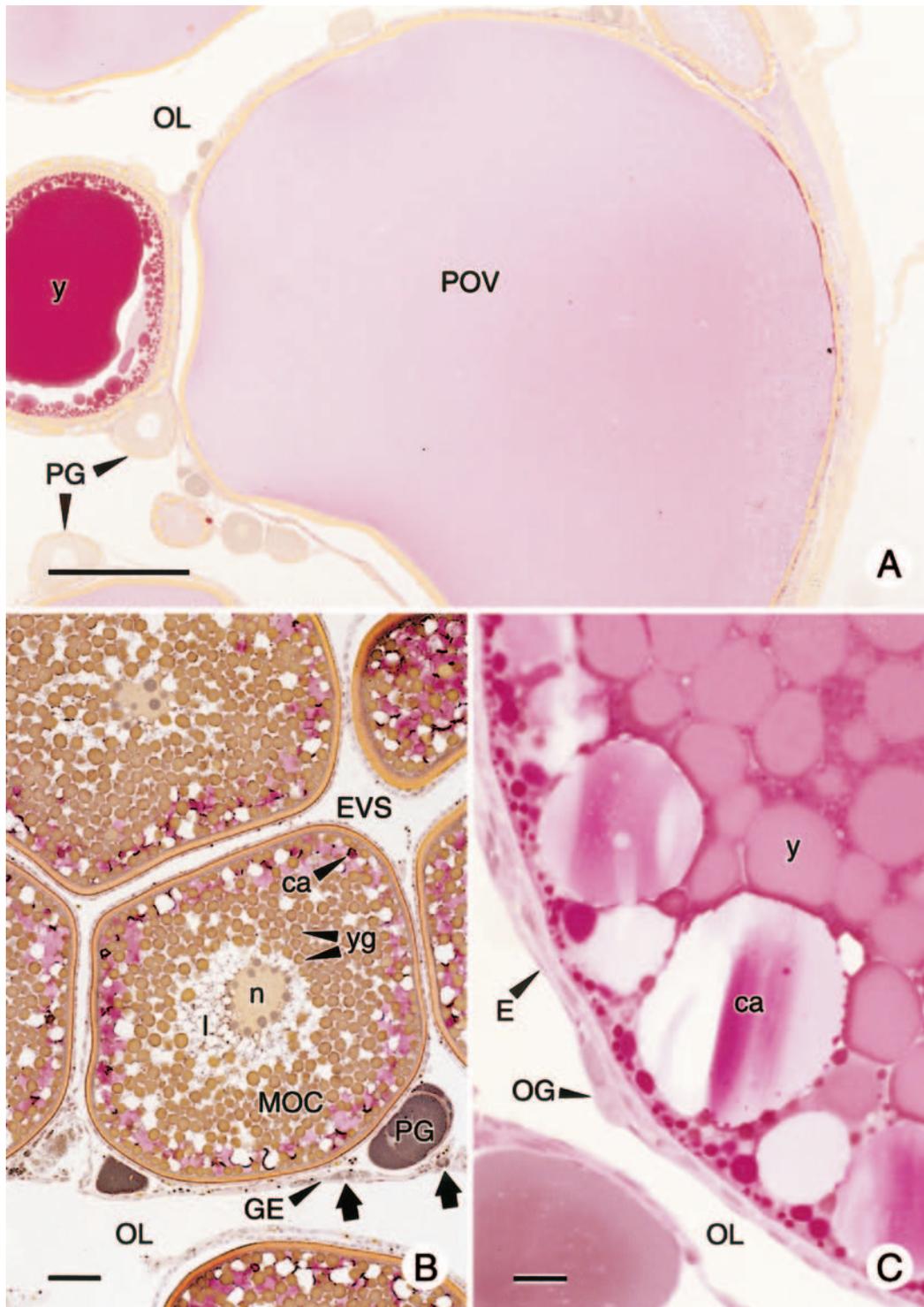


FIG. 4. A. Periphery of the ovary in *Hemiramphus brasiliensis* with a preovulatory oocyte (POV) whose periodic acid Schiff-positive (PAS+), protein yolk is lighter-staining than the protein yolk in an oocyte that is not preovulatory. In both oocytes, the protein yolk (y) is fluid. The germinal epithelium separates these two oocytes, and primary growth oocytes (PG), from the ovarian lumen (OL). Bar = 50 μm . B. In the ovary of reproductive *Mugil cephalus*, the germinal epithelium (GE) separates the ovarian lumen (OL) from the stroma in which a prominent extravascular space (EVS) is observed. Within the germinal epithelium, two small primary growth oocytes (arrows) are observed. Larger primary growth oocytes (PG) and mature oocytes (MOC) are surrounded by the EVS. Mature oocytes have globular protein yolk granules (yg) rather than fluid yolk. These become fluid during final oocyte maturation, just before ovulation. Lipids (l) are circumnuclear in position, and cortical alveoli (ca) are peripheral. Bar = 50 μm . C. Portion of a mature oocyte from the Everglades pygmy sunfish, *Elasmoma evergladei*. The content of cortical alveoli is periodic acid Schiff-positive (purple). Globular yolk (y) is also periodic acid Schiff-positive, particularly the intensely-staining, small yolk globules at the oocyte periphery. The germinal epithelium, separating the oocyte from the ovarian lumen (OL), is composed of epithelial cells with flattened nuclei (E) and an oogonium (OG). Bar = 10 μm .

the ovarian germinal epithelium in the protogynous swamp eel, *Synbranchus marmoratus*, produces follicles during the female phase of the reproductive cycle. During sex reversal, however, the same germinal epithelium produces the initial male germ cells before lobules are formed (Lo Nostro and Grier, 2002). This observation introduces a new approach to study of the mechanism of sex reversal in other protogynous species, for example in the perciform families Serranidae, Labridae, and Gobiidae.

Our investigation reveals that there is constant origin of ovarian follicles from the germinal epithelium among taxa. Terminology should reflect this proposal of homology. The histology text book definition of a follicle (see Grier and Lo Nostro, 2000) precisely reflects its origin from a germinal epithelium. The follicle is composed of the germ cell, the oocyte, and surrounding follicle (granulosa) cells that originate from the epithelial (somatic) cells of the germinal epithelium. The follicle is surrounded by a theca, derived from the stromal compartment of the ovary (Grier, 2000) and is always separated from this compartment, throughout development and final oocyte maturation, by a basement membrane. As a basement membrane separates an epithelium from the underlying lamina propria or supportive tissue, so it also separates the follicle from the theca. The follicle basement membrane is derived from that underlying the germinal epithelium (Grier, 2000). The term "follicle complex" (Grier and Lo Nostro, 2000) has been proposed to include the follicle, basement membrane, and the theca, including its blood vessels. The various definitions of a follicle within the fish literature are based primarily on function rather than form, however. These definitions of a follicle include the surrounding theca, with (Redding and Patiño, 1993; Sullivan *et al.*, 1997) or without (Tyler and Sumpter, 1996) the basement membrane.

Terminology may be confusing due to our ignorance of comparative morphology, the use of synonyms, the still-emerging physiological and molecular events causing oocyte growth, the now documented role of a germinal epithelium in follicle formation and in sex reversal (vide supra), and an array of egg types in fishes that is only dealt with superficially herein. The term "final oocyte maturation" has not become "irrelevant and misleading," as argued by Patiño and Sullivan (2002). Final oocyte maturation is used commonly to describe changes in oocytes leading to ovulation observed in common snook (Neidig *et al.*, 2000) and other marine fishes (*viz.*, Brown-Peterson *et al.*, 1988, 2002) and is embedded in the fisheries literature. Oocyte changes leading to ovulation include cytoplasmic and nuclear events (as in Patiño and Sullivan, 2002), and, in our opinion, these events mark final oocyte maturation prior to ovulation. All of the changes in follicles as they mature can be viewed as maturation or growth.

Definitions based on homology of form can aid in understanding reproduction across a broad array of

taxa. For example, mammals have an advanced follicular morphology not found in teleosts. As above, the teleost follicle consists merely of the oocyte and its encompassing, monolayer of follicle (granulosa) cells, defined as a "primordial follicle" (*viz.*, Van Blerkom and Motta, 1979). In mammals, however, the follicle cells divide, become many cells deep, surround the oocyte to produce a primary follicle. Then, a fluid-filled space develops, to form the antrum (Gray *et al.*, 1995) of a tertiary follicle. From the standpoint of comparative anatomy, the fish ovarian follicle corresponds to the mammalian "primordial follicle," and all oocyte maturation occurs within the homologue of the mammalian "primordial follicle." Furthermore, as in fish (Grier, 2000), mammalian follicles originate from a germinal epithelium (Zamboni, 1972), *i.e.*, they are homologues. Recent studies of mammalian reproduction confirm that follicle cells in sheep develop from cells of the germinal epithelium (Heywood *et al.*, 2002), and that a possibly active germinal epithelium is present in mice (Johnson *et al.*, 2004: fig. 2). We anticipate that additional homologous characteristics within reproductive systems across a broad array of taxa are yet to be revealed.

The annual reproductive cycle is hypothesized to be the source of morphological variation among testis types. Five reproductive classes have been described in males of the common snook, *Centropomus undecimalis* (see Taylor *et al.*, 1998), seatrout, *Cynoscion nebulosus* (see Brown-Peterson, 2003), the cobia, *Rachycentron canadum* (see Brown-Peterson *et al.*, 2002), the swamp eel, *Synbranchus marmoratus* (see Lo Nostro *et al.*, 2003), and the freshwater goby, *Pseudogobius bonelli* (see Cinquetti and Dramis, 2003): regressed, early maturation, mid maturation, late maturation, and regression. These are identified by the alternation of the germinal epithelium between continuous and discontinuous types and the stages of germ cells present (Grier, 2000).

The transition between basal and neoteleosts is marked by numerous physiological and morphological modifications, such as type 4 tooth attachment (Fink, 1981) and acellular bone (Parenti, 1986), characters that *Esox* shares with neoteleosts (Parenti, 1986; Johnson and Patterson, 1996). Teleost evolution was described as paedomorphic by Fink (1981) because some characters observed in adults of more advanced teleosts, such as tooth attachment mode, approximate the early developmental stages of primitive teleost fishes. The evolutionary transition from an anastomosing tubular to a lobular testis could have resulted from the elongation of the testis germinal compartments during the early maturation class when the testis enlarges prior to the breeding season. The process could have evolved as a simple change in formation of the supporting basement membrane of the germinal epithelium. Similarly, restriction of spermatogonia to the distal ends of lobules in the atherinomorphs is mirrored in perciforms by the establishment of distal epithelioid cords of Sertoli cells and spermatogonia in cobia testes

during the regression and regressed classes of the annual reproductive cycle (Brown-Peterson *et al.*, 2002). Clusters of spermatogonia become established during the same reproductive classes in common snook (Grier and Taylor, 1998). During regression in these perciforms, but not in atherinomorphs, spermatogonia also become established along the walls of lobules. There is a marked change in the arrangement of Sertoli cells in atherinomorphs compared to other fishes. Sertoli cells extend processes across the lobules, and a lobule lumen is absent. We hypothesize that the difference in the way in which Sertoli cell processes bridge the widths of the lobules, as in *Fundulus grandis* (Fig. 1A) and *Hemiramphus brasiliensis* (Fig. 1C), prevents the colonization of the lateral lobule walls by spermatogonia—they are only observed at the distal termini of the lobules. Evolution of the atherinomorph testis type, the strongest evidence supporting atherinomorph monophyly (*viz.*, Parenti, 2004), is hypothesized to entail mechanisms that prevent the repopulation of spermatogonia along lobule walls during regression and when regressed. Atherinomorphs may be said to have a “regressed” testis that undergoes a functional maturation.

There is scant information on the mechanism of lobule elongation during gonad maturation between spawning seasons, and practically nothing is known about the process of regression. It has recently been proposed that fish testes shift between meiosis-dominated to mitosis-dominated cell divisions during their annual reproductive cycles (Grier, 2002). The supply of spermatogonia, from which meiotic germ cells are derived, becomes progressively exhausted between early maturation, mid maturation, and late maturation. It has not been appreciated that in the latter part of the annual reproductive cycle, especially regression, the testes are actively preparing for the next reproductive cycle, *i.e.*, the lobules become repopulated by spermatogonia. The same is true in the teleost ovary, at least in *Centropomus undecimalis*, in which the process of folliculogenesis was interpreted using ovaries from regressed fish (Grier, 2000). There is significant cell division during the regressed class in *Synbranchus marmoratus* that involves both Sertoli cells and spermatogonia (Lo Nostro and Grier, 2003). Sertoli cells have been demonstrated to divide in the atherinomorph *Poecilia latipinna* (see Grier, 1993: fig. 25), and the wrasse, *Thalassoma bifasciatum* (see Koulish *et al.*, 2002). Mitotic cell division in fish testes during the regression and regressed classes, as defined by Taylor *et al.* (1998), has hardly been investigated. In light of growth processes during these classes, however, Brown-Peterson *et al.* (2002) suggested that the term “resting stage” no longer be used. Active cell division likely takes place in fish gonads throughout the annual reproductive cycle, even when not spawning.

There appear to be two locations within the testes of perciforms from which spermatogonia are derived: in common snook (Grier and Taylor, 1998) and cobia (Brown-Peterson *et al.*, 2002), clusters or elongation

of the lobules composed of spermatogonia and Sertoli cells become established at the distal ends of lobules. Divisions of these cells result in lobule elongation (Grier, 1993). Lobule growth was inferred to occur via a branching process; in common snook, anastomosing testis morphology results from fusion of lateral lobule walls (Grier and Taylor, 1998). We have not observed anastomosing of the lobules in any atherinomorph.

Spermatogonia also repopulate the lateral walls of testicular lobules. By the end of the breeding season, the population of spermatogonia is exhausted, these cells having formed sperm. There are always scattered spermatogonia along the lobule walls that apparently do not divide, however. They compose a “discontinuous germinal epithelium,” one that spans reproductive seasons and are the *prima facie* evidence for a permanent germinal epithelium in fishes (Grier, 1993; Lo Nostro *et al.*, 2003). During the regression and regressed classes—the mitosis dominated classes—spermatogonia within the discontinuous germinal epithelium become mitotically active, repopulate the lateral lobule walls and form a continuous germinal epithelium, again composed of spermatogonia and Sertoli cells. The term “epithelioid” was first applied to a description of the testes in the cobia in reference to cords of spermatogonia and Sertoli cells that grow from the distal termini of the lobules during the regressed class in the annual reproductive cycle (Brown-Peterson *et al.*, 2002). Because they lack a lumen, these cells do not fulfill the criteria that define an epithelium (Grier, 2000; Grier and Lo Nostro, 2000). The epithelioid arrangement of cells at the distal termini of lobules is transitory. When a lumen develops, these cells compose a germinal epithelium.

Changes in the male germinal epithelium, used to define annual reproductive classes first in common snook (Taylor *et al.*, 1998), cannot be used to define annual reproductive classes in females. The basic difference between the sexes that prevents this simple characterization is the early initiation of meiosis in females. In common snook, the germinal epithelium is active throughout the year, producing follicles where the oocyte is in arrested meiosis, diplotene of the first meiotic prophase, and the germinal epithelium is always discontinuous. In males, only diploid spermatogonia persist in the regressed class and the germinal epithelium is continuous, becoming discontinuous during mid maturation and late maturation. The annual alternation between continuous and discontinuous germinal epithelia in the male teleost germinal epithelium permits the use of changes to be used for defining annual reproductive classes.

These proposals emphasize that morphological change within the testis during the annual reproductive cycle can lead to the formation of distinct testis types. Interestingly, *Schindleria*, a gobioid fish that has been characterized as “. . . the most radically ontogenetically truncated fish” (Johnson and Brothers, 1993, p. 469), reportedly has a restricted lobular testis (Thacker and Grier, 2004). This corroborates our hypothesis that

arrest of the late maturation-regression phases leads to formation of the atherinomorph testis type; that is, it is like the paedomorphic testis of mature adult *Schindleria*.

Testis type provides another way to distinguish among model fish organisms: the zebrafish, *Danio rerio*, has an anastomosing tubular testis, whereas the atherinomorph medaka, *Oryzias latipes*, has a restricted lobular testis (Table 1). We predict that the fugu, *Takifugu rubripes*, a derived percormorph, has an unrestricted lobular testis. Our proposal is that simple changes in the annual reproductive cycle have resulted in these different testis types. The genetic basis of these differences is unknown. Identifying morphological variation, and proposing the source of that morphological variation, is a first step in understanding underlying genetic mechanisms.

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