# EVOLUTIONARY ATTEMPTS AT 4 EYES IN VERTEBRATES*

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## ABSTRACT

*Purpose*: To understand and compare the optical, histological, and ecological differences among 4 vertebrate species that have had evolutionary attempts toward 4 eyes.

*Methods*: An evolutionary attempt at 4 eyes is defined as the duplication of one or more structures integral to the refraction or interpretation of the visible spectrum for that animal. We reviewed and compared the known optics, histology, and ecology of each of these vertebrate species with attempts at 4 eyes including *Anableps anableps*, *Dialommus fuscus*, *Mnierpes macrocephalus*, and *Bathylychnops exilis*. These animals have developed portions of ancillary eyes that have diverged from the primary globe in 3 different patterns. At least 1 specimen of each of these vertebrate species known to have 4 eyes was examined histologically and compared to the animal's ecology and current cladistic relationship.

*Results*: *A anableps* has 2 distinct optical systems in each eye: an upper one for aerial vision and a lower system for aquatic vision. These systems feature separate retinae and an asymmetric lens to achieve focus in the aerial and aquatic vision, but only 1 optic nerve per eye. The visual system is split horizontally to function optimally in a "prone" position in the water. *D fuscus* is a terrestrial feeder and has a vertically (almost perpendicular to the long axis of the fish) divided cornea using pigment and a condensation of collagen as the divider, a single pupil, and a divided retina. The split cornea allows for the fish to remain vertical with 1 cornea in air and 1 cornea in water. *M macrocephalus* is probably closely related to *D fuscus* with a similar split cornea. *B exilis* is a mesopelagic inhabitant living at approximately 200 to 1,000 m and has an ancillary globe that "buds" off the primary globe. This secondary globe is directed inferiorly toward the ocean floor as compared to the primary globe, which is directed 35º superiorly from the horizontal. Adult species of *B exilis* have 2 additional scleral bodies suspected to be lenses. If so, these structures would be capable of focusing light from the inferior field onto the superior retina, presumably adding to the panoramic inferior visual field. There are other mesopelagic species, including *Styleophorus chordatus*, *Opisthoproctus grimaldii*, *Scopelarchus gantheri* (or *guentheri*), *Dolichopteryx binocularis*, *Benthalbella infans*, and *Evermannella indica*, that have other unusual ocular mechanisms, such as retinal diverticulae and lens pads capable of reflection, but do not meet the definition of multiple eyes, as defined for purposes of this work.

*Conclusions*: *D fuscus* and *M macrocephalus* are terrestrial feeders requiring aquatic and aerial vision, and hence have a split cornea for this purpose, and they probably use their anterior corneae for terrestrial vision. *A anableps* swims at the surface with combined aerial and aquatic vision for feeding and protection from predators. *B exilis* is a mesopelagic feeder requiring a binocular visual field in the horizontal meridian and above, and simultaneously is a bottom scavenger using an ancillary globe and perhaps scleral lenses for recognition of bioluminescent detritus.

Although 2 of these models are related (*D fuscus* and *M macrocephalus* ), these 4 fish represent 3 separate, distinct, and unrelated convergent evolutionary attempts toward 4 eyes in vertebrates satisfying the ecological needs of each. The 3 different models are unrelated evolutionarily and are found in 3 separate orders.

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## INTRODUCTION

Evolutionary attempts to produce 4 eyes in vertebrate species is defined as the duplication of one or more structures integral to the refraction or interpretation of the visible spectrum for that animal.

The capacious cauldron of evolution has created at least 3 attempts at 4 eyes in vertebrates. There are at least 3 distinct, completely different evolutionary attempts at providing 4 different species of fish with 4 eyes. Each of these fish has evolved 4 eyes that are functionally advantageous in their own ecological niche. Understanding evolutionary attempts at providing multiple eyes will provide further grist for the understanding of the speed and the potential plasticity of ocular evolution and will suggest pathways of investigation to determine phylogenetic relationships.

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## METHODS

Specimens representing each of the 4 species were obtained from collections of major oceanography institutes. Thanks to the Los Angeles Museum of Natural History and to Richard Fenney for the juvenile *B exilis* (LACM 9528-11); thanks to Cynthia Klepadlo and Scripps Institute of Oceanography in La Jolla, for the specimens of *Styeophorus* (specimen #SIO77-171), *Dolichopteryx* (specimen #SIO77-157), and *Macropinna microstoma* (specimen #SIO71-65); and thanks to Douglas Markle and William Pearcy of the Oregon State Department of Fisheries and Wildlife for the adult *Bathylychnops exilis* (specimens #OS12443 and #OS14840). The specimens of *Dialommus fuscus* (CAS #201890) and *Mnierpes macrocephalus* (CAS #2138855) were generously loaned by John McCosker, PhD, from the California Academy of Sciences, and the Steinhart Aquarium. *Anableps anableps* was obtained from a local commercial fish merchant and was observed alive for 24 hours and then euthanized with eugenol as the liquid anesthetic. These specimens were measured and photographed and 1 eye was enucleated, or in the case of the OSIO specimen, a previously enucleated specimen was examined histologically. The specimens were stained with hematoxylin and eosin, examined, and compared with each other and previous reports. The evolutionary relationships and cladistic position of each species were examined and compared with one another.

## RESULTS

The vertebrate evolutionary attempts at 4 eyes can be divided into at least 2 categories: (1) epipelagic (3 species with 2 different models) and (2) mesopelagic (1 species with 1 model).

### EPIPELAGIC

#### *Anableps anableps*

There are 3 species of *Anableps*, including, *A anableps*, *A dowi*, and *A microleis*. The ecology of these species differs, but each has similar environmental needs for multiple eyes, and the eyes are virtually the same. Hence, description of a single representative eye will suffice to cover all 3 species.

The eye of *A anableps* is spherical and shows a double cornea divided horizontally, parallel with the long axis of the animal. This cornea is part of 2 distinct optical systems, as has been previously described[1] (Fig 1). The aerial cornea appears steeper (radius of curvature not measured) than the aquatic cornea. In light-adapted conditions, the aerial pupil is larger than the aquatic pupil. The iris displays a finger-like projection that divides the pupillary aperture into halves (Figs 2 and 3). The division of the iris corresponds to the pigmented band on the cornea, creating a pupillary aperture for the aerial image separate from the similar pupillary aperture for the aquatic image. In a light-adapted state, the anterior flap extends to overlap the posterior flap (Fig 3). Previous investigators have found that with dark adaptation, the pupil dilates to form a single dumbbell-shaped aperture.[2]

FIGURE 1

*Anableps anableps* as seen swimming with 1 cornea above and 1 cornea below waterline. Note that pupil is parallel with waterline.

Histologically, the cornea is thicker at the boundary between the upper and lower pupillary aperture corresponding with the waterline while swimming. The lens is oval and egg-shaped (pyriform) with the more rounded circular portion of the lens noted on the ventral half of the eye corresponding to the aquatic portion of the globe (Figs 4 and 5). The lens diameter is less through the aerial pupillary aperture than the diameter of the lens along the visual axis through the underwater aperture.[3] Hence, the surface of the lens is less curved at the aerial visual axis than at the underwater aperture.

FIGURE 2

*Anableps anableps* pupil with overlapping edges. Note that overlap is asymmetric. In right eye, posterior portion of pupil overlaps anterior portion of pupil, but in left eye, the reverse is true.

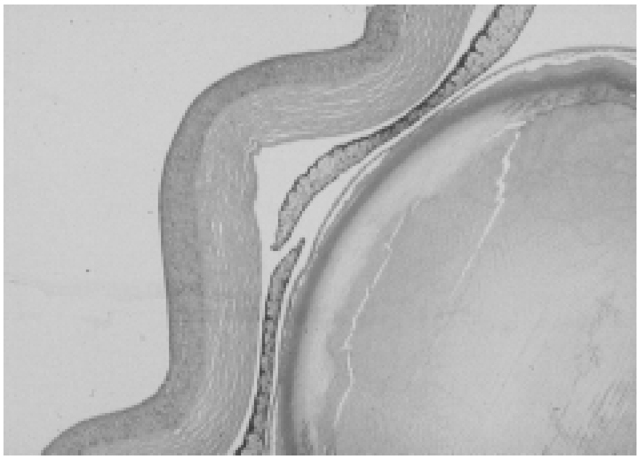

**FIGURE 3**
Histologic photographs of *Anableps anableps* showing large round crystalline lens, with pupillary margins overlapping. Corneal ridge is not an artifactual change (hematoxylin-eosin, x40).

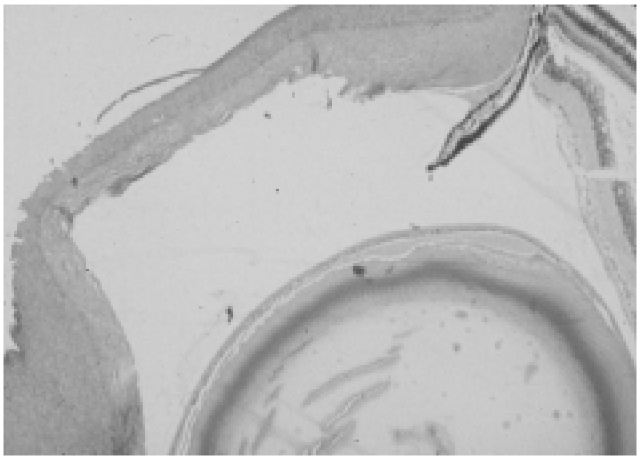

**FIGURE 4**
Anterior segment of *Anableps anableps.* This view represents aerial view of eye. Note more flattened curve of lens as compared with the inferior aquatic cornea in Fig 5. Note corneal ridge at lower left of figure (hematoxylin-eosin, x40).

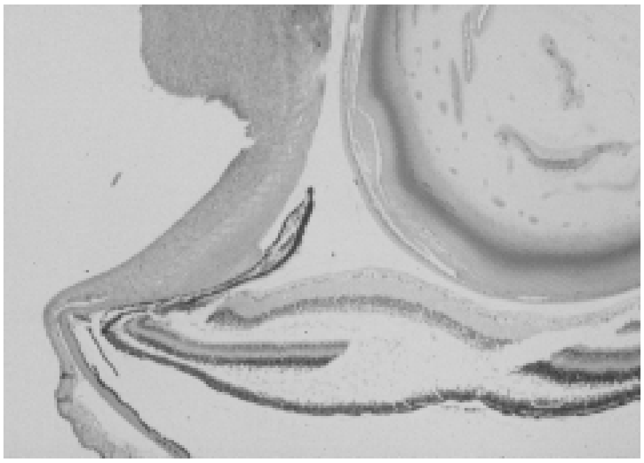

**FIGURE 5**
Anterior segment of *Anableps anableps.* This view represents aquatic view of eye. Note the more steeply curved lens as would be encountered through the inferior corneal window. Note corneal ridge, dividing cornea into an upper and lower half, in the upper left of figure (hematoxylin-eosin, x40).

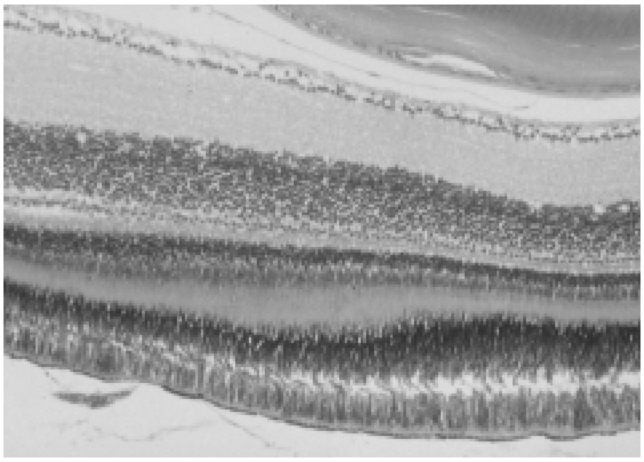

**FIGURE 6**
Inferior half of retina of *Anableps anableps* that subserves aerial image with an increase density of inner nuclear layer. Note the long photoreceptors. Edge of lens can be seen at top of photograph (hematoxylin-eosin, x100).

In the retina, the inner nuclear layer in the ventral portion is thicker and contains more bipolar cells (Figs 6 and 7). There are a greater number of ganglion cells in the lower retina, with perhaps one half to two thirds as many in the dorsal retina. The retinae are distinct in that there is a separation between the retina that subserves the aquatic cornea and lens as compared to the inferior-positioned retina that subserves the aerial retina (Fig 8). The ganglion cell concentration diminishes as the retina approaches the division between the ventral and dorsal retina (Figs 6 and 7). There is a single optic nerve that receives projections from each separate retina.

### *Mnierpes macrocephalus and Dialommus fuscus*

The eyes of *M macrocephalus* and *D fuscus* are very

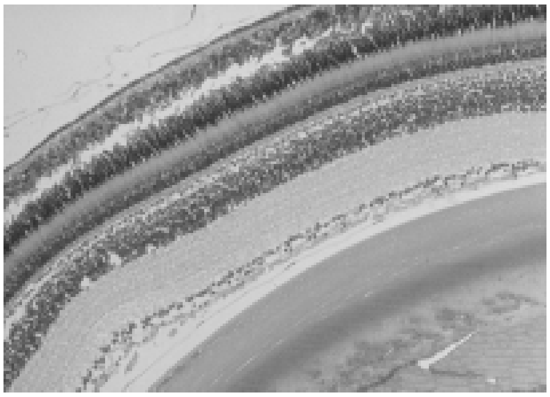

**FIGURE 7**
Superior half of retina of *Anableps anableps* that subserves the aquatic image from inferior cornea. Note density of inner nuclear layer that is about one half or two thirds of inferior retina as seen in Fig 6. Lens can be seen at bottom of figure (hematoxylin-eosin, x100).

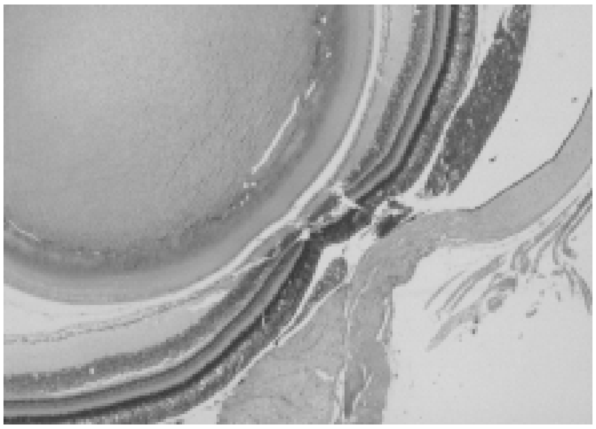

FIGURE 8
Mid portion of eye of *Anableps anableps.* Note narrowing of retina as it approaches midline. Note that lens is very close to retina. Note cartilage in sclera in upper right of figure (hematoxylin-eosin, x40).

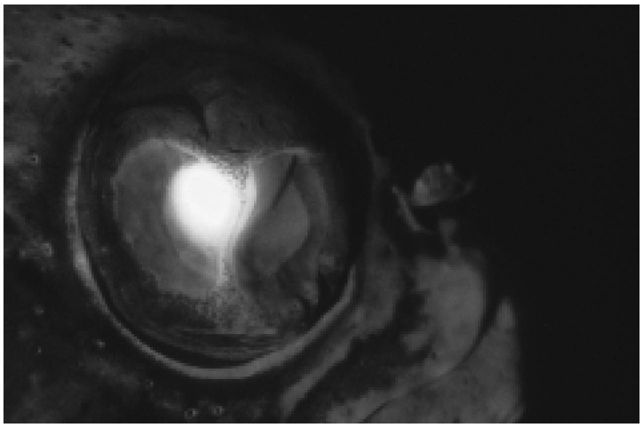

FIGURE 9
External photograph of *Dialommus fuscus.* Note corneal ridge with pigment and corneal condensation.

similar and are probably very closely related. These eyes will be described together, but the differences will be noted as needed.

The eyes are set high on the head and rather close together. Movement of the eyes is described as subtending "through wide angles," although no specifics are given.[4] The cornea is divided vertically by a pigmented stripe that creates a slightly larger anterior window than posterior window (Figs 9 and 10). Previous investigators have described the cornea as flat, but no keratometry or documentation was given.[5] Other fish with aerial and aquatic vision have been documented as having a flat cornea.[6] Our specimen was collapsed from previous fixation, so few comments can be made on the corneal configuration. There is an aphakic space on the rostral aspect of the pupil, creating a horizontally oval pupil set parallel to the mouth opening (Fig 11). The iris color was a dark gray, although in life it is apparently bright blue.[7] These investigators state that the iris color will change from blue to black in response to bright light.[7] Previous investigators describe corneal pigmentation along the dorsal and ventral margins that supposedly reduced the aperture of the pupil,[7] and this was confirmed in our specimens. Our specimens and all photographed specimens have the boundary between the anterior and posterior at an angle of approximately 30º from the vertical and specifically not vertical (Fig 9). This has not been noted and has not been considered in the optical analysis of its ecology.

Histologic examination revealed a circular lens rather typical for teleosts, but unlike *A anableps*, this lens was symmetric. The lens was cataractous in our specimen and probably represented fixation artifact. The distance from the retina was short, suggesting a high index of refraction and a "Matthessien's lens" (the focal length of the lens can be reduced because light is continuously bent within the

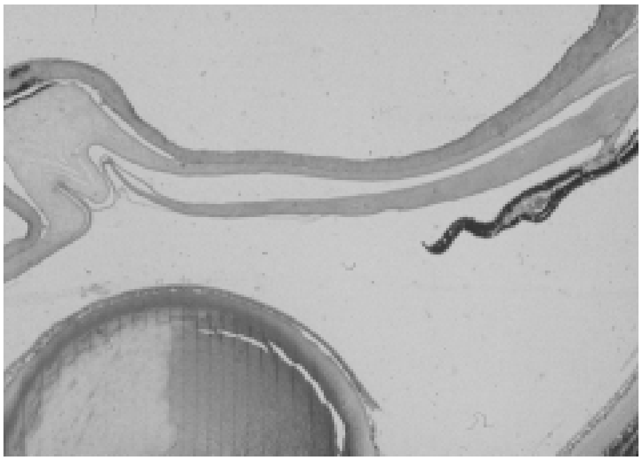

FIGURE 10
Anterior segment of *Dialommus fuscus.* Note corneal ridge at upper left. Crystalline lens is in lower left (hematoxylin-eosin, x40).

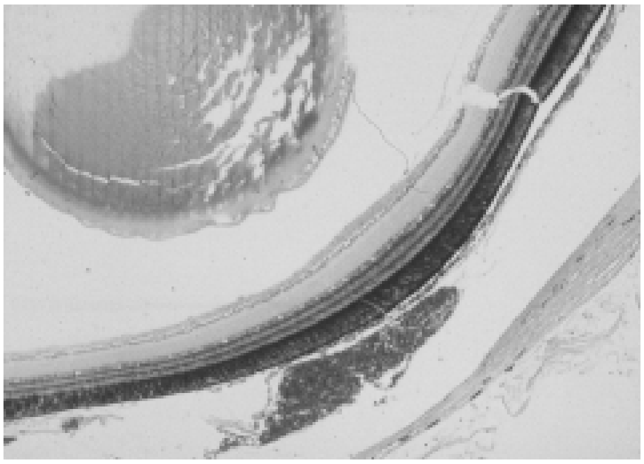

FIGURE 11
Posterior segment of *Dialommus fuscus.* Note that lens is close to retina as is typical for piscine lenses. Note choroidal gland, which is a vascular rete (hematoxylin-eosin, x40).

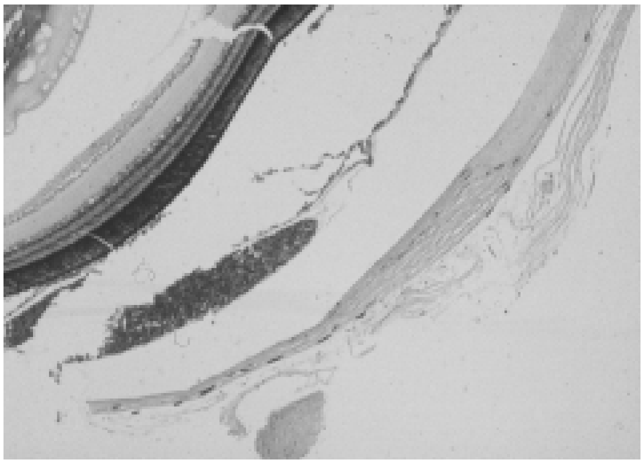

**FIGURE 12**
Posterior segment of *D fuscus*. Note choroidal gland just to left of center and cartilage in upper right (hematoxylin-eosin, x40).

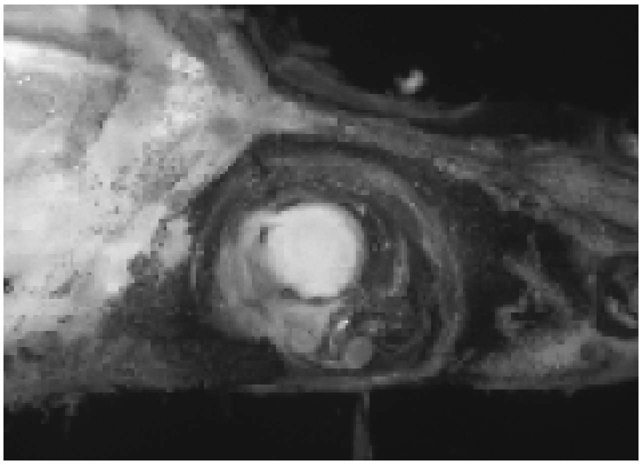

**FIGURE 13**
External photograph of *Bathylychnops exilis*. Note large eyes with a secondary eye budding off primary eye, and 2 scleral lenses.

lens, and the lens can be made aplanatic because the lens has a decreasing gradient of refraction), as is seen in other fish (Fig 12). In contrast to *A anableps*, we could not document that the retina was divided into halves, although a retinal fissure that corresponds to the vertical bar on the cornea creating halves of the globe presumably for the aerial and aquatic portions of the visual field had been described.[5] We were able to note that the concentrations of ganglion cells seem to decrease toward equator and anterior to the equator of the globe. The sclera is lined with cartilage. A choroidal gland was noted (Fig 12). The eye has been described as foveate, and both rods and cones are present.[8,9]

## BATHYPELAGIC

### *Bathylychnops exilis*

The eyes are prominent and occupy 3% (18.5 mm eye and 62 cm body lengths) of the body length and seem to be a predominant feature of the fish. The space between the eyes is narrow. The primary globes gaze upward at about a 30º angle above the horizontal, probably with binocularity and stereopsis (Fig 13). The secondary globe found at the limbus is directed inferiorly and caudally at approximately 40º below the horizontal meridian (Fig 14). The juvenile specimen measured 121 mm and the adult measured 62 cm (Fig 13). The equatorial diameter of each eye of the adult measured 18.5 mm, but the specimen was so collapsed that anterior-posterior measurements were not considered accurate. The corneal diameter of the primary globe was approximately 10.5 mm. The equatorial diameter of the secondary globe was approximately 7 mm. Both the primary and secondary globes are lined with cartilage. The lens of the primary eye appears to be a standard piscine lens, very large and spherical (Fig 15). There

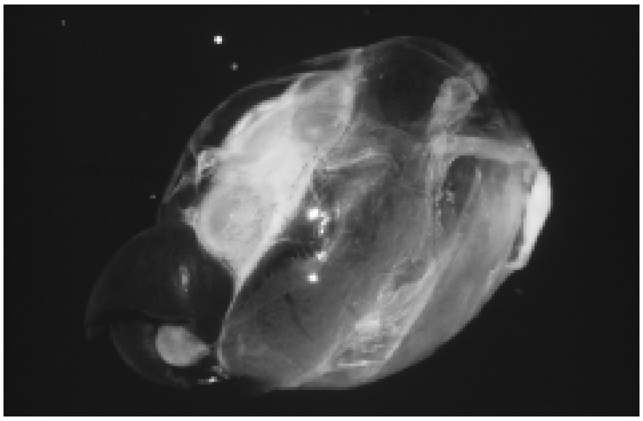

**FIGURE 14**
Enucleated specimen of *Bathylychnops exilis*. Note secondary globe budding off primary globe with a secondary lens. Note 2 scleral lenses.

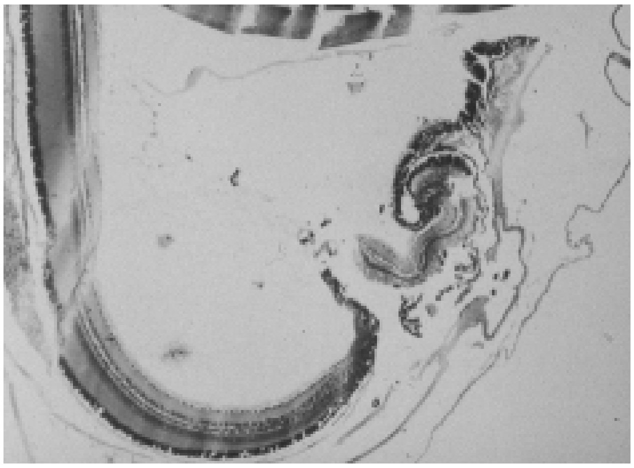

**FIGURE 15**
Whole eye specimen of juvenile *Bathylychnops exilis*. Note large lens as compared to size of eye. Note retinal diverticulum at bottom left (hematoxylin-eosin, x40).

is a thin cornea and a relatively shallow anterior chamber with a thin wispy iris. The secondary globe has no separate cornea but a very thin superficial layer that is probably epithelium. There is a large scleral thickening (compared to the size of the diverticulum) that is approximately 2.5 mm in diameter, but the anatomy is distinct and different from the primary lens. This secondary lens is biconvex and elevated above the surface of the contiguous cartilaginous sclera. The scleral thickening occupies a position in the secondary globe that strongly suggests that it functions as a lens. However, the lens architecture is distinctly different from the lens of the primary globe. On histologic examination, it appeared to be regular and well organized, with a lamellar structure similar to but lacking the typical lens fibers of the primary lens, but softer and of different construction. The retina is a contiguous diverticulum from the primary globe and contains all layers of the retina as seen in the primary globe (Fig 16). There is a small operculum occluding the slit connecting the primary and secondary globes (Fig 17). Where the retinal diverticulum overlies the secondary lens, the retinal pigment epithelium is lost and the retina thins. The thinner retina is represented by 2 layers of epithelial-like tissue and is probably translucent or transparent (Fig 18).

In the ventral sclera of the primary globe, there are 2 masses found caudal to the secondary globe. Each of these 2 masses is externally exposed with a similar overlying layer of tissue as is found over the lens of the secondary globe (Fig 19) and the same histological configuration as the lens found in the secondary globe (Fig 20). These supernumerary lenses are contiguous with the cartilaginous coat of the sclera of the primary globe. The juvenile

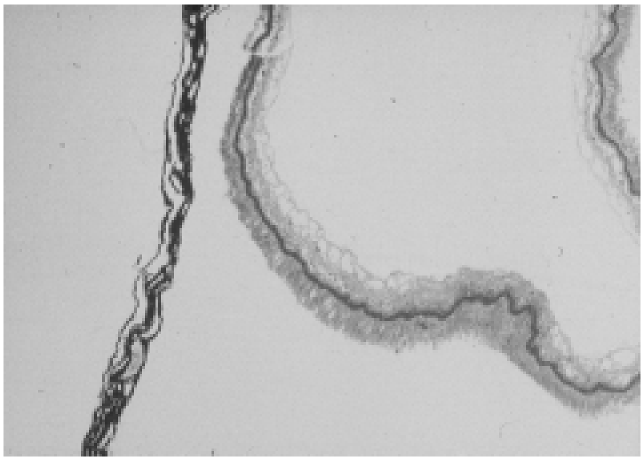

**FIGURE 16**
Retina of diverticulum of *Bathylychnops exilis.* Note long and almost primitive photoreceptors (hematoxylin-eosin, x100).

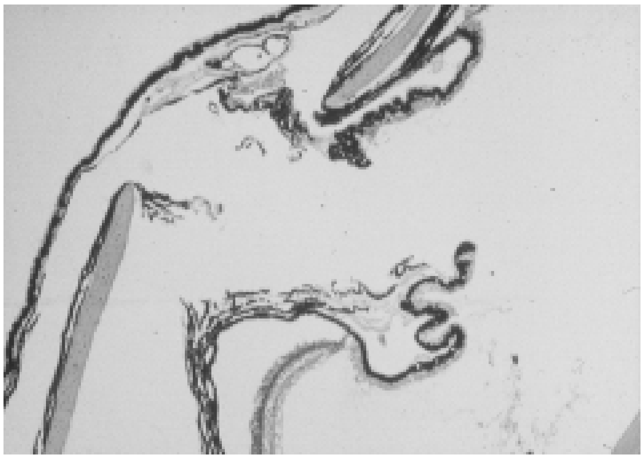

**FIGURE 17**
Portion of secondary eye of *Bathylychnops exilis.* Note operculum in right central portion of figure. Note rounded edges of cartilage on either side of operculum, indicating that cartilage was not broken in processing (hematoxylin-eosin, x100).

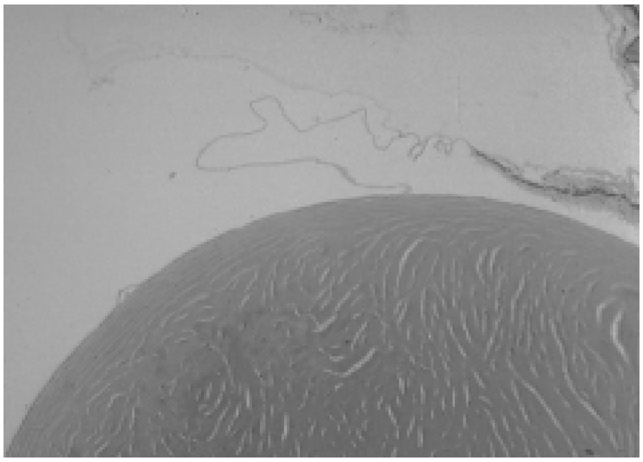

**FIGURE 18**
Lens of secondary eye of *Bathylychnops exilis.* Note difference with primary lens. Retina can be seen on right. As it extends across lens, it thins to 1 or 2 transparent layers (hematoxylin-eosin, x100).

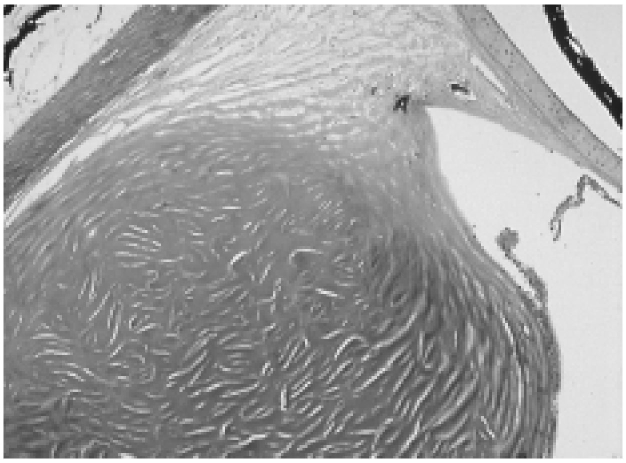

**FIGURE 19**
Supernumerary lens adjacent to secondary globe of *Bathylychnops exilis.* Note cartilage of secondary globe at upper right. Note that secondary lens expands out of perichondrium (hematoxylin-eosin, x40).

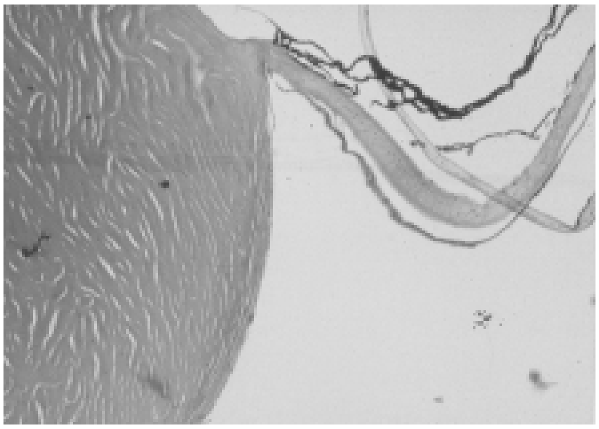

**FIGURE 20**
Secondary lens of secondary eye of *Bathylychnops exilis.* Note that as cartilage comes to edge of lens, it seems to change character into this lens material. Note that the single layer of tissue covering lens is the only tissue between this lens and sea water, as the bottom right portion of the figure is external to the eye (hematoxylin-eosin, x40).

specimen did not appear to have these supernumerary structures, nor a lens for the secondary globe (although this may have been lost in fixation and sectioning), but it did have the aforementioned retinal diverticula, although reduced in size. There was a single optic nerve. The retina appears to have only 1 variety of photoreceptors, and these individual cells appear to be very long, thin, and crowded as is typical for photoreceptors in mesopelagic fish. There appears to be a rather dense inner nuclear layer with a progressive decrease in the number of cells in the inner nuclear and ganglion cell layers. This would suggest a high degree of summation that would be expected in an effort to maximize light collection. A choroidal gland was noted.

### *Cladistics*

A cladogram constructed from currently available information reveals that *A anableps* is unrelated to the other 4 fish and resides in its own order (Cyprinodontiformes) and its own family (Anablepidae). *D fuscus* and *M macrocephalus* are related and probably directly so with the same order (Perciformes) and the same family (Labrisomidae). *B exilis* is also unrelated to all other species in this report being in its own superorder (Protacanthopterygii) and order (Osmeriformes) and family (Opisthoprotidae)[10] (Fig 21).

## DISCUSSION

### *Anableps* (from the Greek, meaning "enlarged eye")

The 3 species of *Anablepidae* have similar ecological needs, although their natural history differs in some respects. These epipelagic fish are intertidal specialists and can be found in fresh, brackish, or even oceanic waters, depending on the species and the individual situation, although they all are principally secondary freshwater fish.

Each of these fish can see simultaneously in air and water with the 2 pupillary apertures and a divided cornea. Finger-like iris flaps between the 2 pupillary apertures prevent double image formation resulting from light refraction at the waterline. There are a dense pigmented stripe and a dense collagen ridge at the midline corresponding exactly to the waterline. The upper cornea and pupillary aperture are for aerial vision, and the lower pupillary aperture is for aquatic vision. The lens is oval in cross section, instead of round, and has the accommodative mechanisms of anterior-posterior movement along

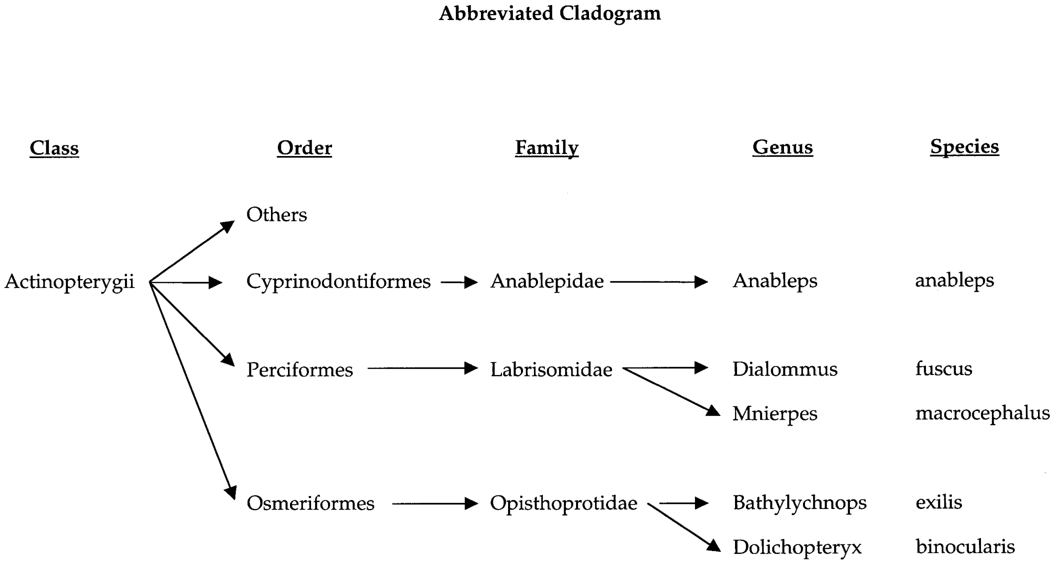

**FIGURE 21**
Cladogram indicating current understanding of relationship of fish order and families to each other.

the axis of the pupil, as do other fish species.[3] The fish maintains itself at the waterline with the horizontal intracorneal strip of pigmentation and the overlapping iris projections creating the dual pupils matching the waterline (Fig 1). Curiously enough, in the right eye the posterior leaf overlaps the anterior leaf of the iris, but in the left eye, the anterior leaf overlaps the posterior leaf (Fig 2). The asymmetry of such pupils is not understood.

The optical structures in *Anableps* allow simultaneous vision in the air and water with 2 different optical axes. Tectal projection of the aerial and aquatic visual field has been mapped by electrophysiologic methods and was found to be similar to that of freshwater fishes except for an area above the waterline with a greatly enlarged tectal magnification in the form of a horizontal band above the waterline in the aerial visual field. This correlates with increased ganglion cell density in the corresponding retinal region.[11] The threshold for movement is lower in the aerial visual field than in the aquatic field and seems correlated with high cone density in the central retina.[11]

A surprising amount of investigation has been performed on this species, revealing that the aerial visual field does have an optomotor response in the aerial field, but not in the aquatic field.[12,13] Mapping the visual system, by recording electrical discharges in the optic tectum in response to a small optic stimulus in the visual field, has demonstrated that the aerial system has better acuity and is more important to the species (Figs 4 and 5).[11,14] The ventral retina, subserving the aerial image, has been shown to be larger and thicker than the dorsal retina (Figs 6 and 7). Investigators have also determined that there is approximately double the number of cones in the ventral as compared to the dorsal retina.[15,16] The egg-shaped lens and divided retina, allowing for simultaneous vision above and below the waterline, have also been documented.[3,15] Other investigators have speculated that the optomotor response was located in the optic tectum, and if so, they believe that the mechanism itself was the result of lateral inhibition.[13] The iridial extensions create dorsal and ventral apertures on the cornea so that the ventral part of the retina views through a dorsal aperture directly into the air with the associated corneal and lenticular adaptations to compensate for the refractive consequences of different optic media (Figs 2 and 3). Unquestionably, this fish has evolved to have simultaneous aerial and aquatic vision. The retina consists of rods, single cones, and 2 different classes of double cones. The cones have visual pigments with maximum absorbencies of 409 nm, 463 nm, and 576 nm. Clear oil droplets (better termed ellipsosomes) are noted in the inner segment of some of the cones.[17]

Evolutionarily, the *Anableps* species are between the Ostariophysi and the Perciformes. They are neither primitive nor particularly advanced. They are in the order Cyprinodontiformes, and are viviparous, although not all members of this order are. This small specialized family of toothcarps, Anablepidae, contains only 3 species, *A anableps* (mainly brackish–eastern South America), *A microlepis* (North and northeast coast brackish and oceanic–eastern South America), and *A dowi* (Pacific coast, Central America). These species have morphologic and distribution differences, but their ophthalmic morphology is quite similar. All 3 species resemble flat, floating torpedoes with large eyes. The long axis of the body is parallel with the surface of the water. *Anableps* grows as large as 14 inches long. All 3 species, but especially *A dowi*, eat insects above and below the waterline. *A dowi* will even be an active nocturnal hunter pursuing prey on mud flats out of water.[18] These fish will feed above the water surface, at the water surface, and in the water column. They will leap out of the water to attack aerial insects and use their aerial vision for this purpose.[19] All 3, but especially *A anableps* and *A microlepis*, will feed on the tidal flats, sifting through the mud with unusual teeth acting as a sieve. The aerial vision with the superior cornea and superior half of the lens provides for excellent acuity used to locate prey and to alert the species to predators (birds and small aquatic predators). Since the creature has no lids, it will frequently submerge the aerial eye to wet the surface and to wet its gills. All 3 species probably rely on their aerial vision for protection against predators as a primary function and feeding as a secondary function.

In the 3 *Anableps* species, we have an animal that has filled and occupied a specialized niche requiring excellent aerial vision for feeding but especially for protection against predators. These 3 species probably emphasize their special adaptation differently, with *A dowi*, using its aerial vision more for feeding than its 2 cousins, but all 3 rely heavily upon the adaptation for protection.

### *Dialommus fuscus and Mnierpes macrocephalus*

*Dialommus fuscus* and *Mnierpes macrocephalus* are 2 related fish with similar mechanisms in the evolutionary attempt at 4 eyes. *M macrocephalus* (class Actinopterygii, order Perciformes, family Labrisomidae, tribe Mnierpini, genus Mnierpes) is a fish known from the brackish and fresh water intertidal zone of the west coast of the Central and South American continent (Panama, Columbia, and Equador). *M macrocephalus* and *D fuscus* are the only 2 members of this tribe (tribe Mnierpini, subfamily Labrisominae). *Dialommus fuscus* (tribe Mnierpini, genus Dialommus), first described by Gilbert in 1891, is known only from the Galapagos Islands and is in the same tribe and closely related to *M macrocephalus*. The Galapagos Islands, no stranger to evolutionary oddities, have allowed this species to develop as a distinct species quite apart from *M macrocephalus*, from which it probably evolved.

Presumably, samples of *M macrocephalus* were simply exported to the Galapagos some number of millions of years ago, being transported on the currents from the west coast of northern South America to the Galapagos, where speciation occurred. Nevertheless, the attempt at 4 eyes probably preceded that journey, and as a result, the 2 eyes of these 2 species resemble each other closely. The adaptive advantage of the dual imaging is demonstrated by its retention following the isolation of *D fuscus*. Both *M macrocephalus* and *D fuscus* are considered “rockskippers” or “mudskippers” and as such can be considered with a few other fish that are amphibious, although none of the other “mudskippers” has such an adaptation. These 2 fish have been minimally studied, and few histologic specimens of *M macrocephalus* are known. Only 1 is known of *Dialommus*, although few details are given.[20]

Both species occupy similar ecological niches. Both are oviparous. *M macrocephalus* applies its eggs to vertical rock faces in its territory, and *D fuscus* is assumed to do the same. Both are intertidal and will actively shuttle back and forth between rock pools and a terrestrial environment. Both are omnivores but prefer to feed on small crustaceans and other invertebrates. *D fuscus* has been seen as far as 30 m out of water and is surprisingly fast on land.[20,21] These fish measure approximately 15 cm maximum as an adult. They lack swim bladders but can maintain themselves out of water for at least 20 minutes by hyperventilating prior to leaping from tide pools onto the adjoining rock surfaces.[22] These marine fish usually hang on a vertical rock face with the long axis of their bodies perpendicular to the water’s surface, quite the opposite of *A anableps*, which resides parallel to the surface. When the fish is ready to leap out of the water, it will expose the cornea so that the waterline corresponds to the pigmented corneal stripe.[22] Only one investigative attempt has been made to understand the optics, and it is incomplete in some respects. Investigators reported that the corneal halves were set at a 100° angle to one another, producing a flat prismatic surface, and if this is so, they probably possess little refracting power, although this has not been confirmed.[5,22] Other investigators also believe that the cornea of *M macrocephalus* is flat and solves the optical problem of myopia when the fish is out of water.[5,7,8] The optics of the eye have been modeled by the same investigators but not measured in vivo. Their model predicted that the species would have a “clear” image with both the aerial and aquatic cornea while in water and, when out of water, would have a “double” image through the prismatic cornea. Few data are given to support this conclusion. Behavioral observations would suggest that this species has very acute near vision, since they consume small invertebrates at close range. Behavioral observations suggest that these species have excellent distance acuity as well, since movement from considerable distance will frighten the animal. These limited investigations suggest that this animal uses its anterior facing cornea for near acuity and foraging and, presumably, this anterior cornea for predator avoidance, since birds would be a threat. Accommodation is presumably obtained by direct movement of the lens in an anterior-posterior fashion, as is typical for most fish.[3,5]

The optics of the eye of these 2 species must be interesting, although convincing evidence of the model is lacking. We may consider other fish as examples to understand these species. For example, the lens of the mudskipper *Periophthalmus* is slightly flattened to correct for the convergence refraction of its strongly curved cornea in air.[23] *Anableps* uses a pyriforme lens positioned to focus simultaneous images from both the aquatic and the aerial focal planes, but both *M macrocephalus* and *D fuscus* have round lenses, as confirmed by several specimens including those for this work. At least 2 investigators believe that these species have 2 flattened corneal surfaces and a central corneal ridge.[5,8,9] With the powerful highly refractive round lens that is typical for fish and is present for this species, the adaptation of a flattened cornea would facilitate acute aerial vision and would represent a rare adaptation to solve this visual problem. The flattened surfaces are supported at the center of the cornea by a narrow, pigmented vertical thickening of the cornea. The dorsal and ventral margins of the cornea are heavily pigmented along with the opaque vertical thickening, reducing the aperture of the eye to the anterior and posterior corneal windows, creating pseudopupils. This flattened cornea would allow for accommodation in air by anterior-posterior movement of the lens as seen in other fish.[3,4] The adaptation of a flattened cornea is demonstrated in other fish, such as the “flying fish” (*Cypselurius heterurus*),[6] but has not been documented by any measurement in *M macrocephalus* or *D fuscus*. Previous dissection and histologic examination reveals that the retinal fissure is positioned directly opposite the vertical corneal bar and that a fusiform process is absent. In 1942, Walls[24] wrote that if the corneal aperture is reduced by pigmentation, as it is in *D fuscus*, the horizontal visual field will not be sacrificed by these 2 openings, although he predicted that sensitivity would be reduced. Graham and Rosenblatt,[5] in 1970, said that it is unlikely that the most important function of the pigmentation is to shield the retina from excess light. Rather, they believed the purpose of the pigmentation is to screen the curved parts of the cornea that would produce an unfocused image in air. No evidence is given for this conclusion, and, in fact, this seems questionable. The pigmentation in the cornea of our specimen seemed to be peripheral and larger than the pupil.

Munk, in 1969, redescribed the eye of *D fuscus*. He found that the eye is foveate with both rods and cones but that it did not have a fusiform process. He was unable to determine if the cornea had flattened surfaces but suggested they were probably flat.[8,9] As mentioned, the mudskipper *Periophthalmos* utilizes a flattened lens to avoid myopia when in the air, although in water it is hyperopic.[24] This is not a serious limitation for a fish that is active during periods of low tide and spends high tide in burrows or simply remains above the water most of the time. However, *M macrocephalus* lives at an air-water interface and moves continually from one medium to another. Its observed acute vision in both air and water suggests that the flattened corneal surface probably exists and helps provide emmetropia in both media by relying upon the lens as the accommodative mechanism alone. While that seems plausible, there are few data to support that conclusion. Nevertheless, the behavior of air breathing and "mudskipping" in the intertidal zones exposes these fish to avian predators, and additional risks are present in the aquatic environment, suggesting that ametropia in either media would be hazardous.

### *Bathylychnops exilis* (from the Greek, meaning "deep-lamp-eye")

*B exilis* is an unusual species known only in the last 50 years and only by a few published specimens. It is a mesopelagic species that is only caught on specific deepwater expeditions.[24] It was so named because the extra eye was originally reported to be a photophore.[24,25] The ocular structures were first described in 1964 by Pearcy and associates.[26] This fish was found to have a primary globe with a second, nearly complete, eye budding off the primary globe at the rostral limbus. The species has only rarely been described or discussed since the original description,[27-29] and only one ocular specimen has been described.[26]

*B exilis* is perhaps the most bizarre of this collection of four-eyed fish. This benthic species is also known as the "javelin spookfish." This odd fish is a translucent mesopelagic species with evolutionary adaptations so unusual as to seem unbelievable. Specimens of this fish have been taken from depths as shallow as 60 m to the extreme of 1,000 m. The evidence would suggest that this fish is capable of nocturnal upward migration, especially among juveniles, although the adults may live at depths much deeper than 1,000 m. The species is endemic to the northern Pacific Ocean, and its prey probably consists of small crustaceans or coelenterates. Little is known about the ecology of this fish, although it is known to be a predator and also is believed to be a scavenger of benthic debris. Evidence suggests that this species remains motionless until prey comes within a set range. Then, with short bursts of speed, this opportunistic predator will catch and crush the bioluminescent prey within its mouth.[28] This capture mechanism is predicted in part because of the lack of red muscle, much like certain Antarctic fish. Its body is translucent and may lack hemoglobin or myoglobin as other deep or cold water fish, but this has not been evaluated. This binocular predator probably lies in wait for small bioluminescent prey, such as small shrimp, and strikes swiftly. As a secondary method, the fish probably also feeds on bioluminescent detritus on the ocean floor. The secondary globe would allow for the fish to see any bioluminescence beneath it, but focus may be rudimentary.

Land,[29] in 2000, has concluded, with good supporting evidence, that silhouette detection in the water above a mesopelagic predator would be a much more demanding task than the detection of luminescent debris beneath the animal. The decreasing contrast, while looking upward, weighs heavily on the morphology of vertebrate eyes with increasing depth. He concludes that if the contrast is decreased from 100% to 10%, 100 times more light is required, necessitating a ten-times larger eye.[29] Curiously enough, as Land points out, the task of silhouette detection only becomes more difficult with increasing depth, but the detection of bioluminscence becomes easier with increasing depth because of the decrease or absence of upwelling background light.[29] Land also concludes that the size of any downward-directed eye or eye components can be quite small and inefficient and yet still successfully accomplish the necessary task of luminescence detection at mesopelagic depths.[29]

Unquestionably, the most startling anatomical aspect of this species is a second smaller, ventrally located eye that buds directly off near the limbus of the primary eye (Figs 13 and 14). This second eye has a completely formed lens, an independent retina, analogous to a retinal diverticulum in other species, but interestingly enough, no second cornea to cover this second lens (Fig 20). The ganglion cells project their axons to the primary globe and eventually to the single optic nerve exiting the eye. The retinal diverticulum that lines the secondary globe originates and connects to the primary eye through a small slit in the primary globe. The retinal diverticulum completely lines the secondary globe but becomes thin and transparent where it lies adjacent to the interior of the secondary lens. Thus, light can traverse the lens and stimulate the secondary retina in the diverticulum. Covering the slit between the vitreous cavities of the primary and secondary globes is an operculum that blocks the stray light that might scatter through the smaller secondary eye and enter the primary ocular cavity (Fig 17). However, it strains the imagination to understand how there could be stray light from below scattering into any eye at this depth.

In the adult, the primary eye is directed anteriorly and dorsally at a 35° angle with the perpendicular (Fig 13). This provides a rather large stereoscopic field directed somewhat vertically above the horizontal. The smaller secondary globe is located rostroventrally, directed ventrally and perhaps a bit caudally. In this secondary eye, containing cartilage in the sclera, there is a completely formed lens and an independent retina. In adult specimens, the secondary globe may be as large as one-half the diameter of the primary globe, although this does not appear to be the case in juveniles. It would appear that the secondary globe enlarges absolutely and relatively during maturation.

Additionally, there are 2 dense intrascleral masses caudal to the formed secondary globe almost immediately adjacent to one another (Fig 13). These structures resemble the secondary lens found in the secondary globe and may allow for photoreception from the ventral aspects below the sea floor beneathe the jaw of this animal that would otherwise be a blind spot for the more dorsally focused primary eyes. Although the true function of these lenticular masses is not understood, these may be clear lenses in life, allowing for retinal stimulation from the benthic ocean floor. It would be assumed that these extra intrascleral or supernumerary lenses are capable of focusing light on the superior retina and hence allow for visualization of bioluminescent prey directly beneath the animal. The original description by Cohen[24] in 1957 described these bodies as luminous tissue, although no documentation or mechanism is provided.

The photoreceptors of the retina are believed to be entirely rods, although the visual pigments have not been determined. Presumably, this single photopigment is tuned to approximately 475 nm, as are most known photopigments of fish predators at this depth. Bioluminescence has this frequency, and most predators tune to this by using photoreceptors sensitive to this wavelength. There appears to be a high degree of summation because of the dense nuclear layer and progressive decrease of cells in the inner nuclear and ganglion cell layers, and this would be expected because of the extremely low light levels. Interestingly, no tapetum or argenta has been found.

Neurologically, little is known, but the olfactory lobes and the optic lobes are described as large and bulbous, and the acousticolateralis system is also hypertrophic.[28]

As is seen in many avian species, the septum between the globes is very thin and the other orbital structures are rudimentary, suggesting that evolutionary pressures tend to maximize a relative and absolute globe size. The primary and secondary globes are lined with cartilage, presumably for better support. The binocularity of the fish suggests that it is a deep-water predator, and the fish is reported to be a strong swimmer, at least when caught. Presumably, the secondary globe is used to locate detritus or perhaps avoid predation from below. The scleral lenses may increase the visual field by focusing light from a ventral field and would be capable of finding detritus or bioluminescent prey (as many shrimp may be found at this depth). Nevertheless, the secondary globe and the scleral lenses may also be used for predator detection. The evolutionary implications for this fish and its second pair of eyes are profound. Other closely related bathypelagic fish (*Dolichopteryx brachyrhynchus*) possess retinal diverticulae without a secondary lens.[30] Often fish that possess diverticula have tubular eyes described as "telescopic" to maximize light collection at mesopelagic or bathypelagic depths. Fish closely related to *B exilis* do not have anything resembling a secondary eye, suggesting that evolution can and does produce eyes rather quickly. Nilsson and Pelger,[31] in 1994, provided a pessimistic estimate of the evolutionary time necessary to produce an eye as only a few hundred thousand years. This would suggest that this evolutionary adaptation has occurred within a million years or perhaps even half of that. As would be expected, any advantage conferred by adaptation is conserved by evolution, and it would appear that this second eye has evolved quickly, even if its function is not known.

### CLADISTIC AND ECOLOGIC RELATIONSHIP

Although *M macrocephalus* and *D fuscus* are almost certainly directly related and represent the same morphologic adaptation, the other 2 fish and other 2 models are unrelated, giving 3 separate morphologic models. The epipelagic group have each created duplicate corneae to solve the refraction interface problems created by intertidal or multiple environment specialization. This is predictable, since the cornea is the principal refracting surface in almost any aerial species (as refractive surgeons understand), although not in purely aquatic species. The mesopelagic adaptations of *B exilis* are not unexpected either, since corneal changes in these species would be meaningless, but additional or larger photoreceptors in the form of tubular eyes, or additional lenses as in *B exilis*, would maximize the collection of photons in a limited light environment. Not surprisingly, each of the 3 models has probably evolved independently because of the ecological challenge presented and may be considered environmental edge phenomena.

## SUMMARY AND CONCLUSIONS

We have examined specimens of 4 vertebrate species with evolutionary solutions for the acquisition of 4 eyes. There are 3 different morphologic models represented, in 3 separate orders with no apparent direct evolutionary relationship of one to another. Each of the 3 morphologic models appears to have arisen independently as adaptation to specific environmental requirements, and each of the 3 models represents entirely different solutions to these ecological problems. We can conclude that:

1. There are at least 3 different evolutionary attempts to produce multiple eyes in vertebrates with reduplication of ocular structures.
2. Evolutionary attempts at 4 eyes may appear relatively quickly in the geologic time span, suggesting that eyes themselves are not difficult to modify if environmental pressures exist.
3. Clever solutions to ecological requirements for special niches, often at environmental edges, provide evidence that physiologic optics is rapidly adaptable and relatively plastic.

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## DISCUSSION

DR ROBERT D. YEE. Dr Schwab and his colleagues have given us a very interesting description of adaptations in the eyes of several fish. These adaptations reflect changes in anatomy and physiology that enable the fish to compete effectively in their ecological niches. To understand the benefits of these changes, we should briefly review vision in fish. In general, water is not as good a medium as air for light transmission because of opacities in the water and attenuation of light as the depth increases. The latter probably accounts for the large size of eyes in relation to body size in most true fish, or teleosts. The cornea produces relatively little refraction of light because its refractive index is similar to that of water, and is often flattened to streamline the body. Consequently, the round lens is large, often protruding in front of the iris plane, and has a very high refractive index.

Fish that live near the surface have a limited view of the aerial environment. Light rays from aerial objects that strike the water surface are refracted. However, because of the critical angle of 48.8° in fresh water, light rays from some peripheral objects do not penetrate the water, but are directed along the surface or reflected back into the air. Therefore, the fish has a limited circular view of the aerial environment with objects becoming progressively smaller, dimmer and foreshortened toward the edge of the “window”. Beyond the “window”, the fish sees the bottom mirrored on the water surface. The surface dwelling fish, that Dr Schwab studied, have eyes adapted for simultaneous aerial and aquatic viewing. The eyes have appropriate changes in corneal shape, pupillary aperture, lens shape and retinal location to match the different optical requirements.

Mesopelagic fish that live at 200 to 1000 meters must see above and below themselves. Fish with laterally and dorsally placed eyes have binocular and monocular visual fields. However, they have blind areas behind and below. Bathylychnops exilis, as described by Dr Schwab, has small, ventrally placed, ancillary eyes with lens and retinal structures. These eyes fill in parts of the blind areas.

If 4 eyes are good, why do most animals have 2 eyes, and not 4? Binocular vision has important advantages over monocular vision. Phototaxis refers to motion of an animal toward or away from light. Single celled, Euglena has one light sensitive organelle near the root of its flagellum. It makes alternating turning movements away from a light source in an inefficient, irregular path. The larva of the marine worm, Arenicola, has 2 symmetrical, light receptor organs. Simultaneous comparison of stimulation of the 2 organs allows the larva to move toward the light in a more efficient, straighter path. Two eyes increase the total visual field, allow overlapping of fields, permit binocular vision with superior depth perception, and preserve some vision if one eye is lost..

Some animals have partially developed third eyes. For example, the embryo of the lizard, Lacerta, has dorsal midline parietal and pineal organs, that arise from the neuroectoderm. The parietal organ of a lizard, Anguis fragilis, has lens, pigmented, photoreceptor and ganglion cells. The pigmented prothoracic gland of the pupa of the Cecropia moth lies below a translucent part of the dorsal head segment. Duration of daylight is detected and triggers hormonally mediated transformation into the adult moth. The pineal body persists in humans and helps to regulate physiologic circadian rhythms by secreting melatonin.

Vision is important to an animal's interaction with its environment. In human, visual afferents comprise about 50% of the sensory fibers to the central nervous system. About 40% of the CNS participates in vision or eye movements. However, the animal's body and brain must also perform other essential sensory, motor, cognitive and metabolic functions. Therefore, in most circumstances having 4 eyes is biologically wasteful. Despite their adaptations toward 4 eyes, the fish that Dr Schwab described have only 1 optic nerve for each eye. In addition, he has shown that they are not part of a single phylogenetic chain. Rather, they represent independent adaptations to similar ecological niches.

I would like to thank Dr Schwab and his colleagues for an interesting, thought-provoking presentation and paper.

DR IVAN R. SCWAB. I want to thank Dr Robert Yee for his thoughtful comments regarding my manuscript and presentation. He describes key points that were not discussed in my manuscript. He provides a list of physiological and anatomical differences that have allowed fish to adapt to their environment, including large eyes, large visual fields, and a spherical, highly refractive lens. He also pointed out that these 4 fish described in my manuscript have either adapted the ability to see simultaneously in air and water, or have adapted to an enlarged visual field in a mesopelagic environment.

I do not agree with the point that if 4 eyes were truly better than 2, many more animals would have evolved to 4 eyes. I suspect that it is simply rare to have 4 eyes develop because of the anatomic necessities. These animals are in 4 different Orders and have formed multiple eyes by chance, error and great patience.

Four eyes would be evolutionary and embrologically expensive but would serve dramatic additional benefit, especially in situations where an "edge" phenomenon existed. Specifically, edge phenomena would exist between the air and water interface, as well as at the margin of downwelling light versus the bioluminescence of a bathypelagic depth. Hence, these fish are biologic specialists suited for their specific environmental niche because of these adaptations. These biologic specialists do offer insight into visual development and suggest that the development of visual elements can probably proceed in a relatively short geologic time period. Bathylychnops exilis, in particular, will offer insight on convergent evolution as we further understand the lens proteins in the secondary and supernumerary lenses.

I want to thank Dr Yee for his additional points which fill important gaps in my own manuscript.