

The giant salamanders (Cryptobranchidae): Part A. palaeontology, phylogeny, genetics, and morphology

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Abstract.—The Cryptobranchidae, commonly called the Giant Salamanders, are the largest surviving amphibians and comprise two extant genera, *Andrias* and *Cryptobranchus*. There are three cryptobranchid species, the Chinese giant salamander (*Andrias davidianus*; 180 cm, 59 kg), the Japanese giant salamander (*A. japonicus*; 155 cm, 55 kg), and the North American giant salamander (*Cryptobranchus alleganiensis*; 74 cm, 5.1 kg). Because of their iconic status as the world's largest amphibians and their biopolitical significance, all cryptobranchids are subject to major and expanding initiatives for their sustainable management. Cryptobranchids are biologically similar in many ways; however, within these similarities there are differences in their habitats, diet, size, reproductive behavior and seasonality, fecundity and egg size, paternity, and growth and development. These characteristics are a consequence of their palaeontology, phylogeny, genetics, and morphology. Cryptobranchid conservation genetics reveal the evolutionary significant units (ESUs) toward which conservation and research efforts must be directed to provide genetically competent individuals for rehabilitation or supplementation programs. Knowledge of these scientific fields in concert with cultural, political, and economic factors all contribute to cryptobranchid conservation biology and the formulation of optimal strategies for their sustainable management. However, there has previously been no comparative review of the numerous scientific fields contributing to the knowledge of cryptobranchids, and little peer-reviewed material on *A. davidianus* and *A. japonicus* has been published in English. Here we present the first article in a series about cryptobranchid salamanders, “The giant salamanders (Cryptobranchidae): Part A. palaeontology, phylogeny, genetics, and morphology.”

Key words. Giant salamander, cryptobranchid, palaeontology, phylogeny, genetics, morphology, conservation, sustainable management, Cryptobranchidae

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Introduction

“The giant salamanders (Cryptobranchidae): Part A. palaeontology, phylogeny, genetics, and morphology” is the first of a series of three review articles that have been produced to review the biology and sustainable management of giant salamanders. Although there has been much published on giant salamanders, the information has previously been scattered within articles on each of the three species largely in languages of their biopolitical regions: Mandarin Chinese, Japanese, and English.

To maximize the potential for the sustainable management of these species, the public and scientific community must have access to accurate knowledge about them to direct policy and provide for Internet-based information and news portals. Consequently, “The Giant Salamanders (Cryptobranchidae)” suite of articles, review and discuss a broad range of biological data known for giant salamanders, which have been collected globally by researchers and enthusiasts over a period of four years.

Different authors have made varying contributions to each article depending on their area of expertise. However, due to the complexity of rewriting and contributing to

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Figure 1. A North American giant salamander (*Cryptobranchus alleganiensis*) shows the characteristic morphology of the cryptobranchids; large robust dorso-ventrally flattened head and body, small eyes, thick legs with stubby digits, lateral folds of skin for respiration, and sensory papillae for detecting water movement and prey (laterally flattened tail not shown). *Image and copyright by Ray Miebaum.*

the suite of articles as it has progressed over many years, we have included all authors on all articles. The major contributing authors to “The giant salamanders (Cryptobranchidae): Part A. palaeontology, phylogeny, genetics, and morphology” are Amy McMillan and Paul Hime (genetics), Raul Diaz (palaeontology, genetics), and Paul Hime (phylogeny).

The caudate superfamily, Cryptobranchoidea is one of the most ancient amphibian clades and comprises two families Cryptobranchidae and Hynobiidae, totalling 51 species. The family Cryptobranchidae derives its name from the Ancient Greek, “kryptos” (hidden) and “branchos” (gill), which originally referred to the gills which must be hidden in adults as they lack external gills, unlike most aquatic vertebrates (larvae have external gills). The Cryptobranchidae, or “Giant Salamanders,” are the largest surviving amphibians and comprise two genera, *Andrias* and *Cryptobranchus*. There are only three extant cryptobranchid species, the Critically Endangered, Chinese giant salamander (*Andrias davidianus* Blanchard, 1871), the Near Threatened, Japanese giant salamander (*A. japonicus* Temminck, 1936), and the North American giant salamander (*Cryptobranchus alleganiensis* Daudin, 1803) which exists as two formally named subspecies, *C. a. alleganiensis* and *C. a. bishopi* (Petranka 1998).

The Cryptobranchoidea, along with probably (Larson 2003) the fully aquatic caudate family Sirenidae are exceptional within the Caudata (salamanders) in having the reproductive mode of external fertilization (Duellman

and Trueb 1994). As giant salamanders are the largest amphibians in their respective major biopolitical regions, they are conservation icons, not only for threatened amphibians but also, for the sustainable management of watersheds. Sustainable management requires providing the broadest range of educational material that relates to both public interest and species conservation. This knowledge can then be used by field, conservation breeding, and culturally engaged conservationists, to provide the best technical approaches to species conservation, and provide a background for the required political and financial support.

A critical part of this knowledge is the paleontological history and phylogeny to show a species’ evolutionary significance, and how a species fits into the tree of life; while conservation genetics shows its evolutionary significant units (ESUs) for directing conservation and research efforts. However, there has been no comparative review of the conservation biology of cryptobranchids and associated scientific fields, and little peer-reviewed information of the conservation biology of *A. davidianus* and *A. japonicus* has been published in English.

Here we review “The giant salamanders (Cryptobranchidae): Part A. paleontology, phylogeny, genetics, and morphology” in concert with “The giant salamanders (Cryptobranchidae): Part B. range and distribution, demography and growth, population density and size, habitat, territoriality and migration, diet, predation, and reproduction” and “The giant salamanders (Cryptobran-



Figure 2. Fossil salamanders strongly support an east Asian (red ellipse) origin for the Cryptobranchoidea. The continents were distributed very differently in the Mid-Jurassic (170 MYA) before continental drift moved them to their present locations. However, Eurasia and North America remained in the Northern Hemisphere. By the Late Pliocene (3 MYA) the continents had moved to their present positions. *Image courtesy of palaeos site: <http://palaeos.com/mesozoic/jurassic/midjura.html>. Adapted from Gao and Shubin, 2003.*

chidae): Part C. etymology, cultural significance, conservation status, threats, sustainable management, reproduction technologies, aquaculture and conservation breeding programs, and rehabilitation and supplementation.”

Palaeontology and phylogeny

The Cryptobranchoidea is comprised of the giant salamanders, family Cryptobranchidae (found in China, Japan, and eastern North America), and the Asiatic salamanders, family Hynobiidae (found throughout Asia and European Russia). From fossil evidence in Asia, the evolutionary origins of the Cryptobranchidae extend to at least the Mid-Jurassic (160 million years ago [MYA]; Gao and Shubin 2003), with their fossils later being known from Europe, Asia, and North America. Fossils of more recent cryptobranchids from the Late Eocene (40 MYA) to the Early Pliocene (5.3 to 3.6 MYA) are known from two genera and two or three species from over 30 Eurasian localities (Böhme and Ilg 2003). Molecular and morphological studies strongly suggest an Asian origin for cryptobranchids with subsequent expansions into Europe and North America by the Upper Paleocene (3.6 to 2.5 MYA). The expansion into North America was probably facilitated by the resumption of ice ages creating a land bridge between Asia and North America during the Late Pliocene-Early Quaternary glaciation that started about 2.6 million years ago (Kruger 2008).

This basal caudate salamander family has experienced remarkable morphological stasis throughout its evolution, with ancient and modern Cryptobranchids being morphologically very similar. The Late Oligocene

(23.0 MYA) to Early Pliocene (5.3 MYA) species *A. scheuchzeri* was distributed from Central Europe to the Zaissan Basin on the border of Kazakhstan and China. Vasilyan et al. (2010) considered from fossil and paleoclimatological evidence that both fossil and extant *Andrias* species occur in regions with annual precipitation from 90 to 130 cm.

The monophyly of the Cryptobranchoidea (Hynobiidae + Cryptobranchidae) has not been a point of contention (Gao and Shubin 2003; Larson and Dimmick 1993; Larson et al. 2003; Frost et al. 2006; Roelants et al. 2007; Pyron and Wiens 2011), though the base of the salamander phylogeny, relative to the placement of widely accepted clades, has been contentious for many decades, specifically due to the placement of Sirenidae and the relationship of other paedomorphic taxa (see: Wiens et al. 2005; Vieites et al. 2009). Salamanders have displayed a relatively conserved tetrapod body plan, at least since the Jurassic Period (Vieites et al. 2009). The independently derived paedomorphic morphology (a heterochronic change where sexually mature adults retain several aspects of the larval body plan) displayed by several recognized families, has played a central role in discussions of salamander morphology, and whose morphological characters have been considered to play a substantial confounding role in phylogenetic reconstruction.

Fossil cryptobranchids from the Late Eocene to the Early Pliocene are known from two genera and two or three species from over 30 Eurasian localities (Böhme and Ilg 2003; Milner 2000). Phylogenetic and paleontological evidence suggests an East Asian origin for cryptobranchids by, at latest, the Cretaceous (135-100 MYA),



Figure 3. The Late Oligocene to Early Pliocene (23.0 to 5.3 MYA) species *A. scheuchzeri* was distributed from Central Europe to the Zaissan Basin on the border of Kazakhstan and China. Fossil room II, Teylers Museum, The Netherlands *Andrias scheuchzeri* Oeningen. Courtesy of: http://en.wikipedia.org/wiki/Andrias_scheuchzeri

with subsequent expansions into Europe and North America by the Upper Paleocene (Milner 2000) via the Asian-American interchange (Duellman and Trueb 1994), though an alternate scenario has been proposed but not widely accepted (Naylor 1981). This basal caudate family has experienced remarkable morphological stasis throughout its evolution, with ancient and modern cryptobranchids appearing very similar, and neoteny being present since the time of early salamander origins (Gao and Shubin 2001; Gao and Shubin 2003). *Andrias* are morphologically conservative and their skeletons are so similar that *A. davidianus* has been considered a junior synonym of *A. scheuchzeri* (Westphal 1958).

Currently recognized fossil cryptobranchids include *Chunerpeton tianyiensis* (Gao and Shubin 2003), the earliest crown-group member, *Cryptobranchus* (= *Andrias*?) *saskatchewanensis* (Naylor 1981), and *Piceoerpeton willwoodensis* (Meszoely 1967; described from a single vertebra). *Cryptobranchus guildayi* (Holman 1977) was also described, based on limited samples and whose validity had previously been questioned (Estes 1981; Nickerson 2003), but whose apomorphies have recently been dismissed due to as yet undescribed intraspecific skeletal variation for *C. alleganiensis*, and the misidentification of the ceratohyal, which was actually a sacral rib; this taxon is thus synonymous with *C. alleganiensis* (Bred-

hoeft 2010). *Andrias matthewi* has also been described from Nebraska from a single mandible (Cook 1917; see Estes and Tihen 1964; and Naylor 1981). *Zaissanurus beliajevae* has been described from the Eocene/Oligocene of Mongolia and Russia while *Aviturus exsecratus* and *Ulanurus fractus* have been described from the Paleocene of Mongolia (Gubin 1991; Milner 2000).

Cryptobranchoid salamanders (Hynobiidae + Cryptobranchidae) share several synapomorphies including: high chromosomal counts (Hynobiidae: $2n$ [diploid number] = 40-78 and Cryptobranchidae: $2n$ = 60); extremely large nuclear genomes (Hynobiidae: 15.2-46.5 Gbp [Giga base pairs] and Cryptobranchidae: 45.5-53.8 Gbp) (Gregory 2012. Animal Genome Size Database. <http://www.genomesize.com> [Accessed: 12 June 2012]); presence of a hypoglossal foramen and nerve (Fox 1957; Fox 1959); fusion of the first hypobranchial and first ceratobranchial into a single structure, as well as the fusion of the *M. pubotibialis* and *M. puboischiotibialis* (Duellman and Trueb 1994); and retention of a separate angular bone in the lower jaw (Fox 1954; Fox 1959; Zhang et al. 2006; Vieites et al. 2009). Members of the Cryptobranchioidea display other primitive features such as external fertilization (also present in Sirenidae) and the production of eggs either as paired clusters (hynobiids) or strings (cryptobranchids), with one set from each oviduct (Duellman

and Trueb 1994). Cryptobranchid salamanders are specialized for an aquatic habitat of cold, fast flowing, rocky, and oxygen rich streams (Petranka 2010).

Extensive epidermal folds (with a dense subsurface capillary network) are present along the flanks of the trunk and limbs to increase surface area, serving as a body length “gill” for oxygen exchange, with the lungs thought to function only for buoyancy (Guimond and Hutchison 1973). Larval cryptobranchids have a dorsal tail fin and short external gills as do the majority of transforming salamanders. Adult *Cryptobranchus* maintain a single pair of gill clefts, while all are closed in *Andrias* (Duellman and Trueb 1994; Dunn 1922; Meszoely 1966; Rose 2003). The development of an angular bone and lack of a septomaxilla, lacrimal, and os thyroideum are shared skeletal characters of cryptobranchids (Fox 1954, 1959; Rose 2003), while diagnostic generic differences are the presence of four bones contributing to the border of the naris in *Cryptobranchus* (premaxilla, maxilla, nasal, and frontal), with a lack of the frontal bone contacting the naris in *Andrias* (Dunn 1922; Meszoely 1966). *Cryptobranchus* also fails to resorb the third and fourth ceratobranchials (Rose 2003). Other skeletal and ontogenetic differences can be found in Rose (2003).

Cryptobranchoidea, from genetic inference, are considered to have evolved during the Middle to Late Jurassic (Gao and Shubin 2003; Roelants et al. 2007; San Mauro et al. 2005; Zhang et al. 2005; Mueller 2006; Wiens 2007; Zhang and Wake 2009), while some researchers estimate early Cretaceous (Marjanovic and Laurin 2007; San Mauro 2010). Mitochondrial and nuclear DNA analysis shows the family Cryptobranchidae is a monophyletic group (e.g., Weisrock et al. 2005; Matsui et al. 2008; Zhang and Wake 2009) and that the two genera within this family, *Cryptobranchus* (North America) and *Andrias* (Asia) diverged between the Late Cretaceous to the Paleocene (around 70 MYA; Matsui et al. 2008; Zhang and Wake 2009). The sister taxa *A. japonicus* and *A. davidianus* likely diverged in the Pliocene (about 4.3 MYA) and are considered separate species despite a small degree of genetic differentiation (Matsui et al. 2008). The root of the *Cryptobranchus* mtDNA tree likely lies on the branch leading to the Current, Eleven Point, and New Rivers, and a common ancestor in the southern Ozarks and/or southern Appalachians is hypothesized to have given rise to all other populations, which is consistent with a Pleistocene refuge for this species as ice sheets covered the more northern regions until approximately 11,000 Before Present (BP) (Sabatino and Routman 2009).

In a recent study by Wiens et al. (2005), it was revealed that not simply the “presence” of “paedomorphic” characters, but rather the lack of clade synapomorphic characters were what misled phylogenetic analyses. This plasticity in the development of adult/terrestrial characters has allowed for convergence toward morphologi-

cal/ecological specialization in the larval aquatic environment (which secondarily misleads reconstructions). Variation in the “larval” traits in these groups presents a special problem in that not all paedomorphic traits are shared across all clades/species (Wiens et al. 2005), with cryptobranchids presenting an adult skull more similar to those of other fully transformed salamanders (Duellman and Trueb 1994; Rose 1999; Rose 2003; Wiens et al. 2005).

Early morphology-based systematic studies placed Cryptobranchoidea as sister to all remaining salamanders, with the exception of the Sirenidae which are placed as basal on the phylogeny (Duellman and Trueb 1994). The classic study by Larson and Dimmick (1993), combining both molecular and morphological data, placed Sirenidae as sister to all extant salamanders and the early rRNA molecular dataset of Larson (1991) placed Sirenidae nested within the salamander tree. Current support for the basal placement of Cryptobranchoidea has come from molecular, morphological, and mixed datasets (Gao and Shubin 2001; Gao and Shubin 2003; San Mauro et al. 2005*; Wiens et al. 2005*; Zhang et al. 2005*; Frost et al. 2006; Marjanovic and Laurin 2007; Mueller 2006; Wang and Evans 2006; Roelants et al. 2007; Vieites et al. 2009; Pyron and Wiens 2011; * = subsets of analyses presented these relationships), while the basal placement for Sirenidae has come from morphology and some reconstructed phylogenies comprised of molecular and mixed datasets (Duellman and Trueb 1994; Larson and Dimmick 1993; San Mauro et al. 2005§; Wiens et al. 2005§; § = subsets of analyses presented these relationships).

Recent studies utilizing whole mitochondrial genome sequences (Zhang and Wake 2009) and mitochondrial genome and nuclear sequences (albeit, with limited taxon sampling; San Mauro 2010) placed Sirenidae as sister to all salamander families. San Mauro et al. (2005) placed (Sirenidae + Cryptobranchoidea) as sister to all other extant salamanders based on sequence from the 3' end of *Rag-1*. The characters analyzed (i.e., inclusion or exclusion of reproductive morphology and “paedomorphic” traits) and methodology used for phylogenetic reconstruction have played significant roles in the affecting the output of relationships; for this article we follow the Cryptobranchoidea placed basal on the phylogeny and Sirenidae sister to all other extant lineages (as in Vieites et al. 2009, Roelants et al. 2007, and Pyron and Wiens 2011). Nonetheless, we emphasize that deep salamander relationships are not clearly resolved at present.

Conservation genetics—Species and Evolutionary Significant Units

The basis of conservation genetics is identifying the genetic variation within a clade and within its comprising species, and consequently defining species and their genetic sub-populations in conservation categories as Evo-

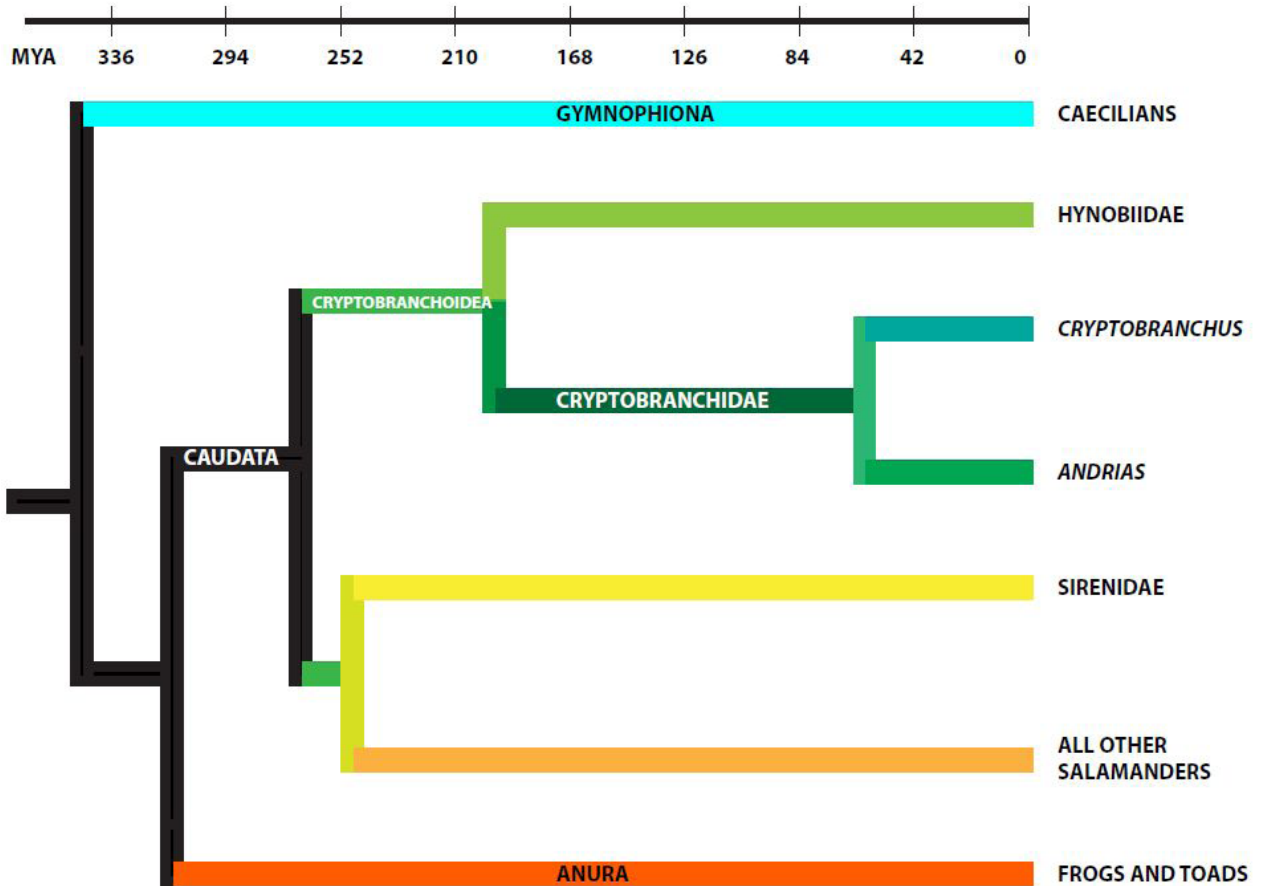


Figure 4. Phylogenetic tree showing ancestry of cryptobranchids and their hypothesized relationships to other amphibians. *Adapted from Roelants et al. 2007.*

lutionary Significant Units (ESU; sensu Wood and Gross 2008). This knowledge in combination with geography defines the range and distribution of species and their ESUs. This knowledge can then be used to perpetuate the genetic variation of the species through a range of practices based on the primary management unit, the ESU. An increasing focus on cryptobranchid conservation, and recent advances in genetic technologies, has resulted in a rapid increase in our knowledge of cryptobranchid conservation genetics.

The molecular techniques used to assess population structure, migration patterns, and their relationship to genetic variation, have rapidly progressed over the last 10 years. This progress has been largely driven by more rapid and cheaper sequencing and computer analysis, Information Technology systems, and a growing bank of molecular techniques and resources (GenBank 2012). Genetic variability in cryptobranchids has been defined with several types of molecular markers including allozymes, mitochondrial DNA (mtDNA) sequencing and



Figure 5 a, b. Taking tissue samples from tail clips (*Image: Amy McMillan*) or blood samples (*Image: Jeff Briggler*) enables conservation geneticists to assess an individual's relationship to other individual cryptobranchids and the relationship of its population to other populations of the same species.

Restriction Fragment Length Polymorphisms (RFLP), Amplified Fragment Length Polymorphisms (AFLP), and microsatellites. Older techniques used to estimate genetic structure and diversity, such as allozyme assays, required sampling whole organisms and may have negatively impacted population numbers. More recent Polymerase Chain Reaction (PCR) based techniques including AFLP, mitochondrial sequencing, and microsatellite markers take advantage of very small amounts of tissues that can be sampled without harm (Tanaka-Ueno et al. 2006).

For example, Foster (2006) collected small amounts of shed blood (amphibian erythrocytes are nucleated) when PIT tags were inserted subcutaneously, or sampled a small tail clip from *C. alleganiensis* that quickly regenerated. Blood samples also can easily be taken from the caudal veins of larger salamanders (see figure 5a). Tanaka-Ueno et al. (2006) found buccal swabbing was the most efficient non-invasive technique for sampling genetic material from caudata. Newer, non-invasive techniques, including environmental DNA (eDNA) sampling, have proven successful for detecting amphibian species in streams (Goldberg et al. 2011) and may prove useful for detection of cryptobranchids in natural habitats (Browne et al. 2011).

Mitochondrial markers have been used to resolve both inter- and intra-specific phylogenetic relationships as well as assess broad-scale population genetic structure. However, mtDNA is maternally inherited and so only tracks female lineages. Polymorphic microsatellite loci are typically found in non-coding or neutral regions within the genomic DNA, and their markers are currently the most commonly used genetic marker for studies of fine-scale population genetic structure in cryptobranchids. However, emerging methods for high-throughput genetic analysis promise to expand the scope of cryptobranchid conservation genetics to a genome-wide scale. Many areas of cryptobranchid research are likely to benefit greatly from ongoing efforts to obtain genome-wide nuclear sequence data, including transcriptome analysis (P. M. Hime, data not shown) and genomic analysis (R. L. Mueller, data not shown) in *Cryptobranchus*.

Polymorphic microsatellite markers can be robust and easily detected on either acrylamide gels or with fluorescence-based detection methods and are available for *Cryptobranchus a. alleganiensis* (Unger et al. 2010), *C. a. bishopi* (Johnson et al. 2009), *Andrias davidianus* (Meng et al. 2008; Yoshikawa et al. 2011), and *A. japonicus* (Yoshikawa et al. 2011). However, as the field of conservation genetics enters the genomic era, genome-wide molecular datasets will become increasingly available for cryptobranchids. These will enable deeper insights into their evolutionary history and cryptobranchid conservation genetics. Through using increasingly sophisticated genetic techniques phylogeny, paleogeography, species status, migration, effective population size, parentage, and population bottlenecks can eventually be known.

Andrias davidianus: Allozyme assays and mitochondrial DNA sequences revealed more variability in *A. davidianus* than in *A. japonicus* (Murphy et al. 2000). Tao et al. (2005) sequenced the mitochondrial control region of *A. davidianus* from the Yangtze, Yellow, and Pearl River regions and found low nucleotide and haplotype diversity within regions, especially the Yangtze River. Both of these studies showed very little differentiation in *A. davidianus* between regions. The population from the Huangshan area in China was genetically distinct from other areas, which suggests localized divergence, probably due to genetic drift and a lack of gene flow between this and other populations (Murphy et al. 2000). Despite the low genetic diversity, Murphy et al. (2000) found substantial substructure among *A. davidianus* populations but poor geographic correlation, even between the three major river systems in China. Nevertheless, Tao et al. (2005) discovered significant phylogeographic differences between the Pearl and Yangtze River regions, and between the Pearl and Yellow River regions. The genetic patterns discovered in these studies suggest that *A. davidianus* have a much higher gene flow between populations than either *A. japonicus* and *Cryptobranchus alleganiensis* (see below). Extensive human-mediated movement of *A. davidianus* may have begun over 3,700 years ago before the advent of historic Chinese Civilization by the Zhang Dynasty (3782-3058 BP; Ebrey 1996); the use of *A. davidianus* for medicine and food may have led to its human mediated transportation and thus may have facilitated this higher gene flow (Murphy et al. 2000).

Andrias japonicus: Early allozyme assays revealed little genetic diversity within *A. japonicus* (Matsui and Hayashi 1992). Mitochondrial DNA sequence variation is also relatively low but nevertheless indicates genetic subdivisions into central and western clades (Matsui et al. 2008). Matsui et al. (2008) noted that the low genetic differentiation in *A. japonicus* contrasted strongly with that of sympatric and also totally aquatic Hynobius species (Cryptobranchoidea). They suggested that the reduced genetic variability in *A. japonicus* may be attributed to polygyny by gigantic males with late sexual maturity and high longevity, a stable aquatic environment as habitat, as well as bottleneck effects during Quaternary glaciations (1.8 MYA to 20,000 BP). They suggested that the low genetic variation of *A. japonicus* may make the species prone to increased risk of extinction. Matsui and Tominaga (2007) found some nuclear genomic diversity in *A. japonicus* in a study of AFLPs but were not able to differentiate any geographic groups not identified with mtDNA methods.

Cryptobranchus alleganiensis: Early allozyme assays revealed very little genetic diversity across the range of *C. alleganiensis* (Merkle et al. 1977; Shaffer 1989). However, mtDNA RFLP and mtDNA sequencing studies revealed enough genetic diversity in *C. alleganiensis* to detect putative clades or management units (Rout-

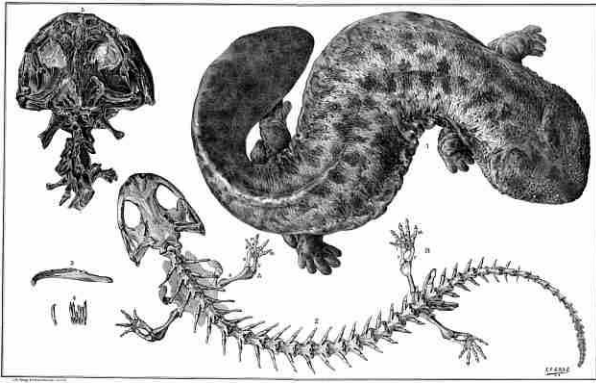


Figure 6. An early figure of Japanese giant salamander, *Andrias japonicus*, showing the dorso-ventrally flattened tail, the very broad head, and massive bulk of the *Andrias* species. The skeleton has remained almost unchanged for tens of millions of years. Image from G. Mösch, *Der Japanische Riesensalamander und der fossile Salamander von Oeningen*, *Neujahrsblatt der NGZH* Nr. 89, 1887. *Cryptobranchus japoniens* Y. de Hoev. (*Japanischer Riesensalamander*.) Nach einer Photographie gezeichnet, in etwas mehr als 1/3 der natürlichen Grösse.

man 1993; Routman et al. 1994; Sabatino and Routman 2009), a finding that was recently supported by nuclear microsatellite DNA markers (Tonione et al. 2011).

The monotypic genus *Cryptobranchus* has traditionally been divided into two distinct subspecies based on morphology and geography. The Ozark hellbender (*C. a. bishopi*) is only found in the Ozark Highlands of Missouri and Arkansas, whereas, the Eastern hellbender (*C. a. alleganiensis*) ranges throughout eastern North America; from eastern New York and Pennsylvania to the north and east, Mississippi, Alabama, and Georgia to the south, and Missouri to the west (Conant and Collins 1998). *Cryptobranchus a. bishopi* is characterized by large dark blotches on the dorsum and dark mottling along the chin, while *C. a. alleganiensis* has small spots on the dorsum and a uniform chin pattern (Petranka 1998). *Cryptobranchus a. bishopi* was described as a separate species by Grobman (1943), but current taxonomy recognizes the Ozark hellbenders as a subspecies.

Recent mitochondrial and microsatellite analyses have shown greater than previously recognized genetic variation in *Cryptobranchus*. These analyses suggest that this group is paraphyletic with respect to the currently recognized subspecies designations, and may potentially harbor unrecognized diversity. However, the species status of genetically distinct entities within this genus has yet to be examined in a comprehensive framework. Crowhurst et al. (2011) used nuclear microsatellite loci to show that *C. a. bishopi* is genetically distinct from *C. a. alleganiensis*, but that within the Ozark region there are two strongly supported groups that are as genetically distant from each other as each is from all *C. a. alleganiensis* samples combined. When the Ozark and Eastern hellbender samples were analyzed separately, the eastern samples resolved as two groups, albeit with weaker sup-

port than the Ozark sample distinction. This finding is not trivial for *Cryptobranchus* conservation. The Ozark subspecies was listed on the US Fish and Wildlife Endangered Species List in November, 2011 (US Government, 2011 No. FWS-R3-ES-2009-0009) and both subspecies have been included on Appendix III of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES).

Work by Sabatino and Routman (2009) using mitochondrial sequencing, and by Tonione et al. (2011) using microsatellite markers, recovered eight independent groups of *C. alleganiensis* which the authors advocated should be treated as separate ESUs. These are the Northern Ozarks, Ohio, and Susquehanna Rivers, Tennessee River, Copper Creek, North Fork of the White River, Spring River, New River, and Current/Eleven Point Rivers. These studies show that gene flow is severely restricted or non-existent among these eight major groups (as measured by the markers under investigation), and potentially among populations (rivers) within groups. Use of highly polymorphic microsatellite markers allows assignment of individual samples to specific management units. For example, Crowhurst et al. (2011) correctly assigned Ozark samples >91% of the time and a new Hellbender population in Georgia had an 84% probability of membership with an adjacent Tennessee River (Albanese et al. 2011).

Morphology and morphometrics

Andrias: The heads of *Andrias* are wide and flat reaching 1/5-1/4 of the snout-vent length. On their heads and necks, *A. davidianus* has paired small tubercles arranged in rows and *A. japonicus* large, single, and scattered tubercles. With both species tubercles are interspersed with abundant tiny sensory neuromasts that detect water movement and the presence of prey (Lannoo 1987). Their snouts are rounded with small nostrils near the snout tip, and their eyes are small and without eyelids. A labial fold is prominent at the posterior of the upper jaw. Their tongue with free lateral margins adheres to the mouth floor. Thick skin folds are present at the lateral side of the body and there are 12-15 costal grooves. All four limbs are short and stout with four fingers and five toes and lack skin folds or prominent interdigital webbing.

Tail length is between 59 and 80% of the snout-vent length. The dorsal fin of the tail is prominent and the ventral fin only conspicuous nears the vent (Fei et al. 2006). Coloration exhibits great variation. The skin of *A. davidianus* is dark brown, black or greenish and *A. japonicus* is reddish-brown with a paler venter; irregularly blotched and marbled with dusky spots (Chang 1936; Thorn 1969). Juveniles often have lighter coloration with small black flecks. Albinos (white or golden) have been recorded (Fei et al. 2006). There is no obvious sexual dimorphism in cryptobranchids, except during the breeding season when

mature males have an enlarged cloaca and females have a swollen belly when gravid (Niwelinski 2007). The larvae of *A. davidianus* have longer gills, their fingers and toes are more pointed, and their color darker than the larvae of *A. japonicus*. External gills disappear when total length reaches 170–220 mm (Fei et al. 2006).

Cryptobranchus: The head is strongly flattened, with small eyes and wrinkled fleshy folds of skin along each side of the body for respiration. Coloration exhibits great variation. The base coloration of *C. alleganiensis* ranges from grayish-black to tan and olive-green across the majority of the body (Nickerson and Mays 1973). The Ozark form *Cryptobranchus a. bishopi*, has many black blotches on the dorsum and the lower lips, while the dorsum of *C. a. alleganiensis* bears black spots rather than blotches, and the throat region may have pale spots (Petranka 2008). Albinos and morphs (orange to red patterns) have been occasionally observed (Dyrkacz 1981; Nickerson and May 1973; Fauth et al. 1996). *Cryptobranchus* retains a single pair of gill slits as adults unlike *Andrias*. Sexual dimorphism (enlarged cloaca in males and swollen belly in gravid female) is only obvious during the late summer to autumn breeding season. The larval stage of *C. alleganiensis* lasts 1–1.5 years during which they grow to 12.5 cm in length, gradually lose their external gills, and develop internal gills and a circular opening on each side to provide water for respiration, as well as development of fleshy fold along the sides of the body for respiration.

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