

Short Note

No remnants of nasolacrimal ducts in any Desmognathini salamanders indicates loss early in their evolutionary history

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Supplementary material

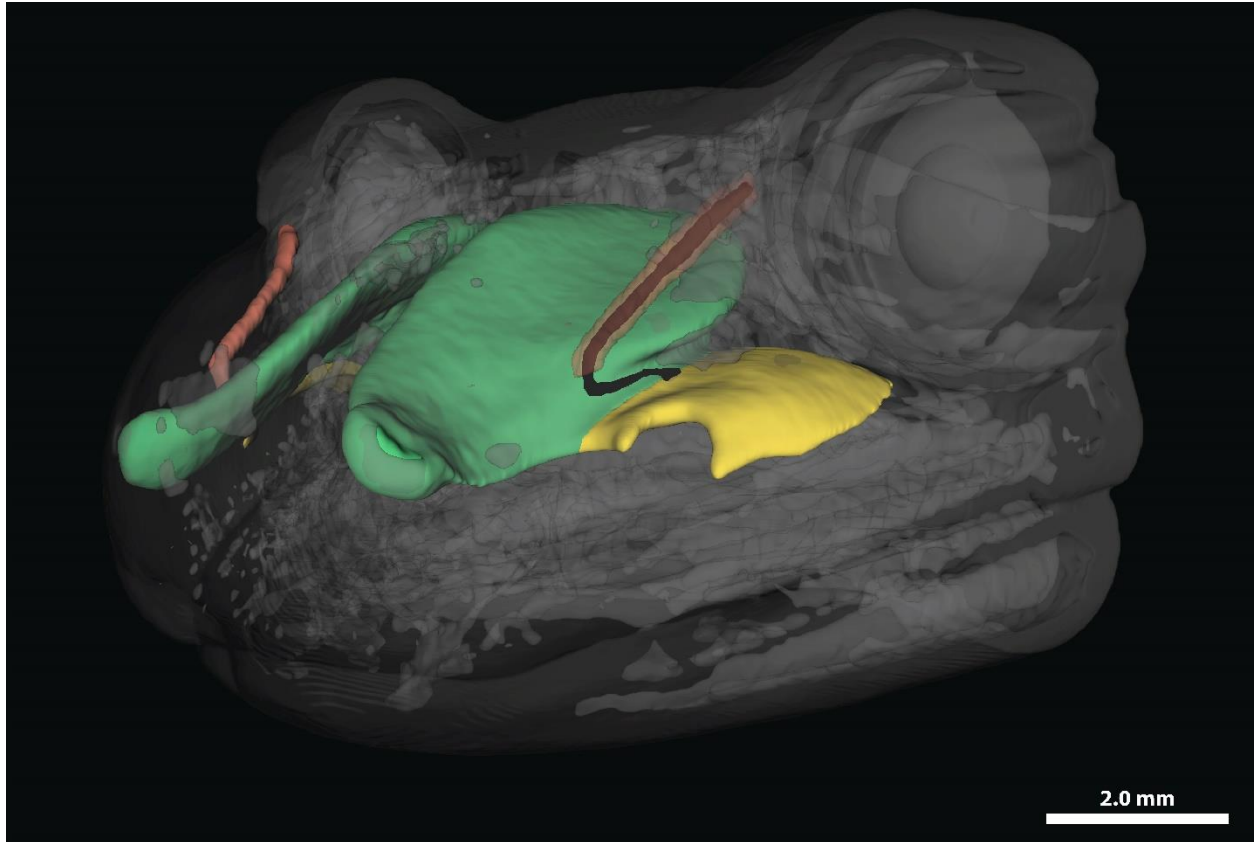


Figure S1. Three-dimensional rendering of diceCT scanned head of *Plethodon shermani*. Nasal cavities were segmented as a green cast, vomeronasal organ cavities a yellow cast, epithelial lining of the nasolacrimal duct a red cast, and lumen of the nasolacrimal duct a black cast. Skeletal and integumentary elements of the whole head cast were highly simplified in 3DSlicer (slicer.org) to allow better visualization of the nasolacrimal ducts, vomeronasal cavities, and nasal cavities.

Appendix S1: Specimens examined

Abbreviations: ASUMZ, Arkansas State University Museum of Zoology; CAS, California Academy of Sciences; DMS, personal collection of David M. Sever (currently held by DSS); DSS, personal collection of DS Siegel; FMNH, Field Museum of Natural History; JYL, personal collection of Jennifer Y. Lamb); MWI, personal collection of Michael W. Itgen; TU, defunct herpetological collection from Tulane University (currently held at Southeastern Louisiana University).

Hemidactyliinae

Batrachosepini: *Batrachoseps attenuatus* (FMNH 278344, FMNH 278345, FMNH 278349, FMNH 278403); **Bolitoglossini:** *Bolitoglossa celaque* (CM 170741), *B. copinhorum* (CM 170766), *B. subpalmata* (FMNH 256094, FMNH 256135, FMNH 256146, FMNH 256150), *Oedipina cyclocauda* (FMNH 179533, FMNH 179559), *Pseudoeurycea bellii* (FMNH 65600, FMNH 65605, FMNH 65609, FMNH 104855); **Spelerpini:** *Eurycea bislineata* (DSS 0506, DSS 0507), *E. chamberlaini* (DSS 0263, DSS 0275, DSS 0277, DSS 0288), *E. longicauda* (DSS 0037, DSS 0039, DSS 0082, DSS 0094), *E. multiplicata* (ASUMZ 4202, ASUMZ 5487, ASUMZ 5495, ASUMZ 6864), *E. quadridigitata* (DSS 0526; from JYL), *E. spelaea* (ASUMZ 4533, ASUMZ 8158, ASUMZ 9356, ASUMZ 9357), *E. tynerensis* [metamorphs] (ASUMZ 18103, ASUMZ 18423, ASUMZ 18424, ASUMZ 18426), *E. tynerensis* [paedomorphs] (ASUMZ 15339, ASUMZ 15359, ASUMZ 22946, ASUMZ 22947), *Pseudotriton ruber* (DMS 1282, DMS 1307, FMNH 247234, FMNH 283054, FMNH 283069, FMNH 283389);

Hemidactyliini: *Hemidactylium scutatum* (ASUMZ 4928, ASUMZ 4930, ASUMZ 5256, ASUMZ 5368).

Plethodontinae

Aneidini: *Aneides aeneus* (FMNH 247570, FMNH 247580, FMNH 274594, FMNH 274598);

Desmognathini: *Desmognathus aeneus* (FMNH 86761, FMNH 86768), *D. auriculatus* (FMNH 87637, FMNH 87638), *D. brimleyorum* (DSS 0520), *D. carolinensis* (FMNH 55769, FMNH 55785), *D. conanti* (DSS 0521, DSS 0522; from JYL), *D. fuscus* (FMNH 86913, FMNH 86979, FMNH 86986, FMNH 87102), *D. imitator* (FMNH 89489, FMNH 89493), *D. monticola* (DSS 0530, DSS 0531; from DMS), *D. ocoee* (FMNH 89118, FMNH 89163), *D. ochrophaeus* (DSS 0534, DSS 0535), *D. orestes* (FMNH 89416, FMNH 89419), *D. quadramaculatus* (DSS 0532, DSS 0533; from DMS), *D. santeetlah* (FMNH 282973, FMNH 282977), *D. welteri* (FMNH 255010, FMNH 255011), *D. wright* (FMNH 247796, FMNH 247800); *Phaeognathus hubrichti* (TU 18004, TU 18005); **Ensatinini:** *Ensatina eschscholtzii* (CAS 13843, CAS 26996, CAS 26997, CAS 217016); **Plethodontini:** *Plethodon albagula* (DSS 0501, DSS 0502, DSS 0514), *P. cadoensis* (DSS 0519), *P. cinereus* (DSS 0504, DSS 0505; from DMS), *P. cylindraceus* (MWI Cyl1–5), *P. dunni* (MWI Dunni1–5), *P. idahoensis* (MWI Idaho1–5), *P. glutinosus* (DSS 0513), *P. metcalfi* (MWI Met1–5), *P. mississippi* (DSS 0523, DSS 0524, DSS 0525; from JYL), *P. montanus* (MWI Mont1–5), *P. ouachitae* (DSS 0517, DSS 0518), *P. serratus* (ASUMZ 5547, ASUMZ 6787, ASUMZ 6842, ASUMZ 29943), *P. shermani* (DMS 1326, DMS 1328), *P. vandykei* (MWI Van1–5), *P. vehiculum* (MWI Vehic1–5).

Rhyacotritonidae

Rhyacotriton olympicus (CAS 47622, CAS 47651, CAS 47679, CAS 47684).

Appendix S2: Collecting permits

Arkansas: *Desmognathus brimleyorum*, *Eurycea multiplicata*, *Plethodon albagula*, *P. caddoensis*, and *P. ouachitae*; Arkansas Game and Fish Commission, permit #012020161 (permit holder: Stanley Trauth).

Illinois: *Eurycea longicauda* and *Plethodon glutinosus*; Illinois Department of Natural Resources, permit #HRP18.6179 (permit holder: Dustin Siegel).

Louisiana: *Desmognathus conanti*, *Eurycea quadridigitata*, *Plethodon mississippi*; Louisiana Department of Wildlife and Fisheries, permit #LNHP-18-011 (permit holder: Jennifer Lamb).

Pennsylvania: *Desmognathus ochrophaeus* and *Eurycea bislineata*; Pennsylvania Scientific Collectors Permit, permit #2017-01-0039 (permit holder: Kenzie Pereira).

Tennessee: *Desmognathus fuscus*; Tennessee Wildlife Resources Agency, permit #1797 (permit holder: Christopher Murray).

MWI collection: *Plethodon metcalfi* was collected from Macon county, NC, and *P. montanus* and *P. cylindraceus* were collected from Avery county, NC, under the wildlife collection license # 18-SC01250 issued by the North Carolina Wildlife Resources Commission. *Plethodon idahoensis* was collected from Shoshone county, Idaho, under the wildlife collection permit #180226 issued by the Idaho Department of Fish and Game. *Plethodon cinereus* and *P. glutinosus* were collected from South Cherry Valley and Oneonta, Otsego county, New York, under the New York State Department of Environmental Conservation scientific collection permit #2303. *Plethodon vehiculum*, *P. vandykei*, and *P. dunni* were collected from Pacific county, Washington, under the scientific collection permit # ITGEN 17-309 issued by the Washington Department of Fish and Wildlife. All permits were issued to M.W. Itgen and the animals were collected between May and August of 2018.

DMS collection: The source of these salamanders and associated collecting permits is unknown. DMS passed away before the writing stages of this manuscript could be completed and we were

unable to find the collecting data from these species, many of which were not tagged. The specimens are currently housed at Southeast Missouri State University in the private collection of DSS.

Appendix S3: GenBank accession numbers

Species	Accession
<i>Aneides aeneus</i>	AY691701.1
<i>Batrachoseps attenuatus</i>	JF449382.1
<i>Bolitoglossa subpalmata</i>	EU275810.1
<i>Desmognathus aeneus</i>	AY691696.1
<i>Desmognathus auriculatus</i>	KR732368.1
<i>Desmognathus brimleyorum</i>	AY691697.1
<i>Desmognathus carolinensis</i>	KR732369.1
<i>Desmognathus conanti</i>	KR732370.1
<i>Desmognathus fuscus</i>	KR732372.1
<i>Desmognathus imitator</i>	KR732373.1
<i>Desmognathus monticola</i>	AY691698.1
<i>Desmognathus ochrophaeus</i>	KR732377.1
<i>Desmognathus ocoee</i>	KR827010.1
<i>Desmognathus orestes</i>	KR827015.1
<i>Desmognathus quadramaculatus</i>	AY650117.1
<i>Desmognathus santeetlah</i>	KR732384.1
<i>Desmognathus welteri</i>	KR732386.1

<i>Desmognathus wrighti</i>	AY691699.1
<i>Ensatina eschscholtzii</i>	AY691702.1
<i>Eurycea bislineata</i>	AY691706.1
<i>Eurycea longicauda</i>	AY650121.1
<i>Eurycea multiplicata</i>	AY691707.1
<i>Eurycea quadridigitata</i>	FJ917633.1
<i>Eurycea spelaea</i>	KY073110.1
<i>Eurycea tynerensis (m)</i>	KF562674.1
<i>Eurycea tynerensis (p)</i>	KF562673.1
<i>Hemidactylium scutatum</i>	AY691711.1
<i>Oedipina taylori</i>	KP900167.1
<i>Phaeognathus hubrichti</i>	AY691700.1
<i>Plethodon albagula</i>	DQ995008.1
<i>Plethodon caddoensis</i>	DQ995013.1
<i>Plethodon cinereus</i>	AY691703.1
<i>Plethodon cylindraceus</i>	DQ995022.1
<i>Plethodon dunni</i>	KF911900.1
<i>Plethodon glutinosus</i>	DQ995027.1
<i>Plethodon idahoensis</i>	DQ995031.1
<i>Plethodon metcalfi</i>	DQ995040.1
<i>Plethodon montanus</i>	DQ995043.1
<i>Plethodon ouachitae</i>	AY691704.1
<i>Plethodon serratus</i>	EU275795.1

<i>Plethodon shermani</i>	DQ995067.1
<i>Plethodon vandykei</i>	AY691715.1
<i>Plethodon vehiculum</i>	AY691716.1
<i>Pseudoeurycea rex</i>	AY650125.1
<i>Pseudotriton ruber</i>	AY650123.1
<i>Rhyacotriton cascadae</i>	AY691694.1