

Ophidiomycosis surveillance of snakes in Georgia, USA reveals new host species and taxonomic
associations with disease

Ellen Haynes^{*1}, Houston C. Chandler², Benjamin S. Stegenga², Laura Adamovicz¹, Emilie
Ospina¹, Dessirée Zerpa-Catanho³, Dirk J. Stevenson^{2,4}, Matthew C. Allender¹

¹ Wildlife Epidemiology Laboratory, University of Illinois at Urbana-Champaign, College of
Veterinary Medicine, Urbana, Illinois, USA

² The Orianne Society, Tiger, Georgia, USA

³ Department of Plant Biology, University of Illinois Urbana-Champaign, Urbana, Illinois, USA

⁴ Altamaha Environmental Consulting, Hinesville, Georgia, USA

***Corresponding author:** haynes11@illinois.edu

Supplementary Table S1: Contingency table showing number of snakes sampled for ophidiomycosis by species and age class in Georgia, USA from 2016 to 2018.

Species	Number sampled	Juvenile	Subadult	Adult
<i>Agkistrodon contortrix</i>	13	2	1	10
<i>Agkistrodon piscivorus</i>	39	8	10	21
<i>Cemophora coccinea</i>	28	5	5	18
<i>Coluber constrictor</i>	57	13	5	39
<i>Coluber flagellum</i>	25	4	7	14
<i>Crotalus adamanteus</i>	25	0	1	24
<i>Crotalus horridus</i>	15	8	1	6
<i>Diadophis punctatus</i>	11	0	0	11
<i>Farancia abacura</i>	27	11	1	15
<i>Farancia erytrogramma</i>	4	1	1	2
<i>Haldea striatula</i>	2	0	0	2
<i>Heterodon platirhinos</i>	35	13	4	18
<i>Heterodon simus</i>	3	0	0	3
<i>Lampropeltis elapsoides</i>	8	2	1	5
<i>Lampropeltis getula</i>	16	0	2	14
<i>Liodytes alleni</i>	4	2	0	2
<i>Liodytes pygaea</i>	15	2	2	11
<i>Liodytes rigida</i>	20	7	6	7
<i>Micrurus fulvius</i>	4	0	1	3
<i>Nerodia erythrogaster</i>	27	7	1	19
<i>Nerodia fasciata</i>	69	25	11	33
<i>Nerodia floridana</i>	2	1	0	1
<i>Nerodia taxispilota</i>	33	8	4	21
<i>Opheodrys aestivus</i>	19	1	2	16
<i>Pantherophis alleghaniensis</i>	61	10	15	36
<i>Pantherophis guttatus</i>	32	6	4	22
<i>Pituophis melanoleucus</i>	9	2	0	7
<i>Sistrurus miliarius</i>	16	1	2	13
<i>Storeria dekayi</i>	2	0	0	2
<i>Storeria occipitomaculata</i>	2	1	0	1
<i>Thamnophis sauritus</i>	19	4	5	10
<i>Thamnophis sirtalis</i>	36	6	9	21
<i>Virginia valeriae</i>	1	0	0	1
TOTAL:	679	150	101	428

Supplementary Table S2: Contingency table showing number of snakes sampled for ophidiomycosis by species and year in Georgia, USA.

Species	Number sampled	2016	2017	2018
<i>Agkistrodon contortrix</i>	13	0	10	3
<i>Agkistrodon piscivorus</i>	39	1	28	10
<i>Cemophora coccinea</i>	28	0	22	6
<i>Coluber constrictor</i>	57	2	44	11
<i>Coluber flagellum</i>	25	1	20	4
<i>Crotalus adamanteus</i>	25	3	20	2
<i>Crotalus horridus</i>	15	3	7	5
<i>Diadophis punctatus</i>	11	0	5	6
<i>Farancia abacura</i>	27	0	8	19
<i>Farancia erytrogramma</i>	4	0	0	4
<i>Haldea striatula</i>	2	0	1	1
<i>Heterodon platirhinos</i>	35	6	18	11
<i>Heterodon simus</i>	3	2	1	0
<i>Lampropeltis elapsoides</i>	8	0	3	5
<i>Lampropeltis getula</i>	16	0	4	12
<i>Liodytes alleni</i>	4	0	1	3
<i>Liodytes pygaea</i>	15	0	3	12
<i>Liodytes rigida</i>	20	0	4	16
<i>Micrurus fulvius</i>	4	0	2	2
<i>Nerodia erythrogaster</i>	27	0	20	7
<i>Nerodia fasciata</i>	69	0	46	23
<i>Nerodia floridana</i>	2	0	1	1
<i>Nerodia taxispilota</i>	33	0	25	8
<i>Opheodrys aestivus</i>	19	0	12	7
<i>Pantherophis alleghaniensis</i>	61	3	35	23
<i>Pantherophis guttatus</i>	32	2	19	11
<i>Pituophis melanoleucus</i>	9	0	6	3
<i>Sistrurus miliarius</i>	16	0	11	5
<i>Storeria dekayi</i>	2	0	1	1
<i>Storeria occipitomaculata</i>	2	0	0	2
<i>Thamnophis sauritus</i>	19	0	5	14
<i>Thamnophis sirtalis</i>	36	0	20	16
<i>Virginia valeriae</i>	1	0	0	1
TOTAL:	679	23	402	254

Supplementary Table S3: Contingency table showing number of snakes sampled for ophidiomycosis by species and month in Georgia, USA from 2016 to 2018.

Species	Number sampled	January	February	March	April	May	June	July	August	September	October	November	December
<i>Agkistrodon contortrix</i>	13	0	0	0	0	1	7	2	1	1	0	1	0
<i>Agkistrodon piscivorus</i>	39	0	0	1	11	6	4	4	4	4	4	1	0
<i>Cemophora coccinea</i>	28	0	0	1	2	8	6	9	2	0	0	0	0
<i>Coluber constrictor</i>	57	0	4	1	15	6	9	9	4	1	3	2	3
<i>Coluber flagellum</i>	25	2	1	4	2	7	0	1	3	1	1	1	2
<i>Crotalus adamanteus</i>	25	2	3	1	0	0	4	2	3	2	1	4	3
<i>Crotalus horridus</i>	15	0	0	0	1	1	2	4	3	4	0	0	0
<i>Diadophis punctatus</i>	11	0	1	0	1	0	3	2	0	0	3	1	0
<i>Farancia abacura</i>	27	0	0	1	7	6	5	5	2	1	0	0	0
<i>Farancia erythrogramma</i>	4	0	0	1	0	1	2	0	0	0	0	0	0
<i>Haldea striatula</i>	2	0	0	0	0	0	0	0	2	0	0	0	0
<i>Heterodon platirhinos</i>	35	0	2	2	9	3	2	2	1	7	6	0	1
<i>Heterodon simus</i>	3	0	0	0	0	0	0	0	0	0	3	0	0
<i>Lampropeltis elapsoides</i>	8	0	0	0	1	2	2	0	1	0	0	2	0
<i>Lampropeltis getula</i>	16	0	0	1	1	7	1	4	1	1	0	0	0
<i>Liodytes alleni</i>	4	0	0	0	1	0	0	2	0	1	0	0	0
<i>Liodytes pygaea</i>	15	0	0	0	2	4	2	3	2	1	1	0	0
<i>Liodytes rigida</i>	20	0	0	0	4	2	4	7	0	2	1	0	0
<i>Micrurus fulvius</i>	4	0	0	0	1	1	0	0	0	0	1	1	0
<i>Nerodia erythrogaster</i>	27	0	0	2	9	9	3	2	1	0	1	0	0
<i>Nerodia fasciata</i>	69	1	2	8	8	3	6	21	10	6	3	1	0
<i>Nerodia floridana</i>	2	0	0	0	0	0	0	2	0	0	0	0	0
<i>Nerodia taxispilota</i>	33	0	0	8	6	5	6	1	2	5	0	0	0
<i>Opheodrys aestivus</i>	19	0	0	0	0	1	5	4	2	2	2	3	0

<i>Pantherophis alleghaniensis</i>	61	0	3	1	13	10	13	5	6	6	1	3	0
<i>Pantherophis guttatus</i>	32	0	1	0	3	7	7	4	4	2	0	3	1
<i>Pituophis melanoleucus</i>	9	0	1	1	3	0	1	1	0	0	0	1	1
<i>Sistrurus miliarius</i>	16	0	0	0	0	0	1	4	8	3	0	0	0
<i>Storeria dekayi</i>	2	0	0	0	0	0	1	0	1	0	0	0	0
<i>Storeria occipitomaculata</i>	2	0	0	0	0	0	0	1	1	0	0	0	0
<i>Thamnophis sauritus</i>	19	0	0	0	6	1	5	4	2	1	0	1	0
<i>Thamnophis sirtalis</i>	36	0	0	0	1	5	7	8	6	3	3	3	0
<i>Virginia valeriae</i>	1	0	0	0	0	0	0	1	0	0	0	0	0
TOTAL:	679	5	18	33	107	96	108	113	72	54	34	28	11

Supplementary Table S4: Contingency table showing number of snakes sampled for ophidiomycosis by species and presence of skin lesions in Georgia, USA from 2016 to 2018.

Species	Number sampled	Skin lesions present	
		No	Yes
<i>Agkistrodon contortrix</i>	13	11	2
<i>Agkistrodon piscivorus</i>	39	26	13
<i>Cemophora coccinea</i>	28	25	3
<i>Coluber constrictor</i>	57	40	17
<i>Coluber flagellum</i>	25	15	10
<i>Crotalus adamanteus</i>	25	18	7
<i>Crotalus horridus</i>	15	14	1
<i>Diadophis punctatus</i>	11	8	3
<i>Farancia abacura</i>	27	15	12
<i>Farancia erythrogramma</i>	4	2	2
<i>Haldea striatula</i>	2	1	1
<i>Heterodon platirhinos</i>	35	30	5
<i>Heterodon simus</i>	3	2	1
<i>Lampropeltis elapsoides</i>	8	8	0
<i>Lampropeltis getula</i>	16	12	4
<i>Liodytes alleni</i>	4	3	1
<i>Liodytes pygaea</i>	15	14	1
<i>Liodytes rigida</i>	20	15	5
<i>Micrurus fulvius</i>	4	4	0
<i>Nerodia erythrogaster</i>	27	11	16
<i>Nerodia fasciata</i>	69	56	13
<i>Nerodia floridana</i>	2	1	1
<i>Nerodia taxispilota</i>	33	18	15
<i>Opheodrys aestivus</i>	19	10	9
<i>Pantherophis alleghaniensis</i>	61	37	24
<i>Pantherophis guttatus</i>	32	29	3
<i>Pituophis melanoleucus</i>	9	5	4
<i>Sistrurus miliarius</i>	16	14	2
<i>Storeria dekayi</i>	2	2	0
<i>Storeria occipitomaculata</i>	2	2	0
<i>Thamnophis sauritus</i>	19	14	5
<i>Thamnophis sirtalis</i>	36	29	7
<i>Virginia valeriae</i>	1	1	0
TOTAL:	679	492	187

Supplementary Table S5: Contingency table showing number of snakes sampled by species and qPCR result for *Ophidiomyces ophiodiicola* in Georgia, USA from 2016 to 2018.

Species	Number sampled	qPCR result	
		Negative	Positive
<i>Agkistrodon contortrix</i>	13	12	1
<i>Agkistrodon piscivorus</i>	39	28	11
<i>Cemophora coccinea</i>	28	27	1
<i>Coluber constrictor</i>	57	50	7
<i>Coluber flagellum</i>	25	25	0
<i>Crotalus adamanteus</i>	25	19	6
<i>Crotalus horridus</i>	15	13	2
<i>Diadophis punctatus</i>	11	10	1
<i>Farancia abacura</i>	27	22	5
<i>Farancia erythrogramma</i>	4	3	1
<i>Haldea striatula</i>	2	2	0
<i>Heterodon platirhinos</i>	35	34	1
<i>Heterodon simus</i>	3	3	0
<i>Lampropeltis elapsoides</i>	8	8	0
<i>Lampropeltis getula</i>	16	11	5
<i>Liodytes alleni</i>	4	4	0
<i>Liodytes pygaea</i>	15	15	0
<i>Liodytes rigida</i>	20	20	0
<i>Micrurus fulvius</i>	4	4	0
<i>Nerodia erythrogaster</i>	27	16	11
<i>Nerodia fasciata</i>	69	62	7
<i>Nerodia floridana</i>	2	2	0
<i>Nerodia taxispilota</i>	33	18	15
<i>Opheodrys aestivus</i>	19	18	1
<i>Pantherophis alleghaniensis</i>	61	53	8
<i>Pantherophis guttatus</i>	32	31	1
<i>Pituophis melanoleucus</i>	9	8	1
<i>Sistrurus miliarius</i>	16	15	1
<i>Storeria dekayi</i>	2	2	0
<i>Storeria occipitomaculata</i>	2	2	0
<i>Thamnophis sauritus</i>	19	18	1
<i>Thamnophis sirtalis</i>	36	33	3
<i>Virginia valeriae</i>	1	1	0
TOTAL:	679	589	90

Supplementary Table S6: Contingency table showing number of snakes sampled for ophidiomycosis by age class and year in Georgia, USA.

Age Class	Number sampled	2016	2017	2018
Juvenile	150	8	89	53
Subadult	101	2	59	40
Adult	428	13	254	161
TOTAL:	679	23	402	254

Supplementary Table S7: Contingency table showing number of snakes sampled for ophidiomycosis by age class and month in Georgia, USA from 2016 to 2018.

Age Class	Number sampled	January	February	March	April	May	June	July	August	September	October	November	December
Juvenile	150	0	1	9	25	9	19	29	11	21	12	11	3
Subadult	101	2	3	4	16	21	15	18	13	3	4	2	0
Adult	428	3	14	20	66	66	74	66	48	30	18	15	8
TOTAL:	679	5	18	33	107	96	108	113	72	54	34	28	11

Supplementary Table S8: Contingency table showing number of snakes sampled for ophidiomycosis by age class and presence of skin lesions in Georgia, USA from 2016 to 2018.

Age Class	Number sampled	Skin lesions present	
		No	Yes
Juvenile	150	126	24
Subadult	101	78	23
Adult	428	288	140
TOTAL:	679	492	187

Supplementary Table S9: Contingency table showing number of snakes sampled by age class and qPCR result for *Ophidiomyces ophiodiicola* in Georgia, USA from 2016 to 2018.

Age Class	Number sampled	qPCR result	
		Negative	Positive
Juvenile	150	144	6
Subadult	101	90	11
Adult	428	355	73
TOTAL:	679	589	90

Supplementary Table S10: Contingency table showing number of snakes sampled for ophidiomycosis by year and presence/absence of skin lesions in Georgia, USA from 2016 to 2018.

Year	Number sampled	Skin lesions present	
		No	Yes
2016	23	20	3
2017	402	305	97
2018	254	167	87
TOTAL:	679	492	187

Supplementary Table S11: Contingency table showing number of snakes sampled by month and qPCR result for *Ophidiomyces ophiodiicola* in Georgia, USA from 2016 to 2018.

Month	Number sampled	qPCR result	
		Negative	Positive
January	5	5	0
February	18	13	5
March	33	18	15
April	107	92	15
May	96	85	11
June	108	97	11
July	113	103	10
August	72	62	10
September	54	47	7
October	34	31	3
November	28	26	2
December	11	10	1
TOTAL:	679	589	90

Supplementary Table S12: Contingency table showing number of snakes sampled for ophidiomycosis by month and year in Georgia, USA.

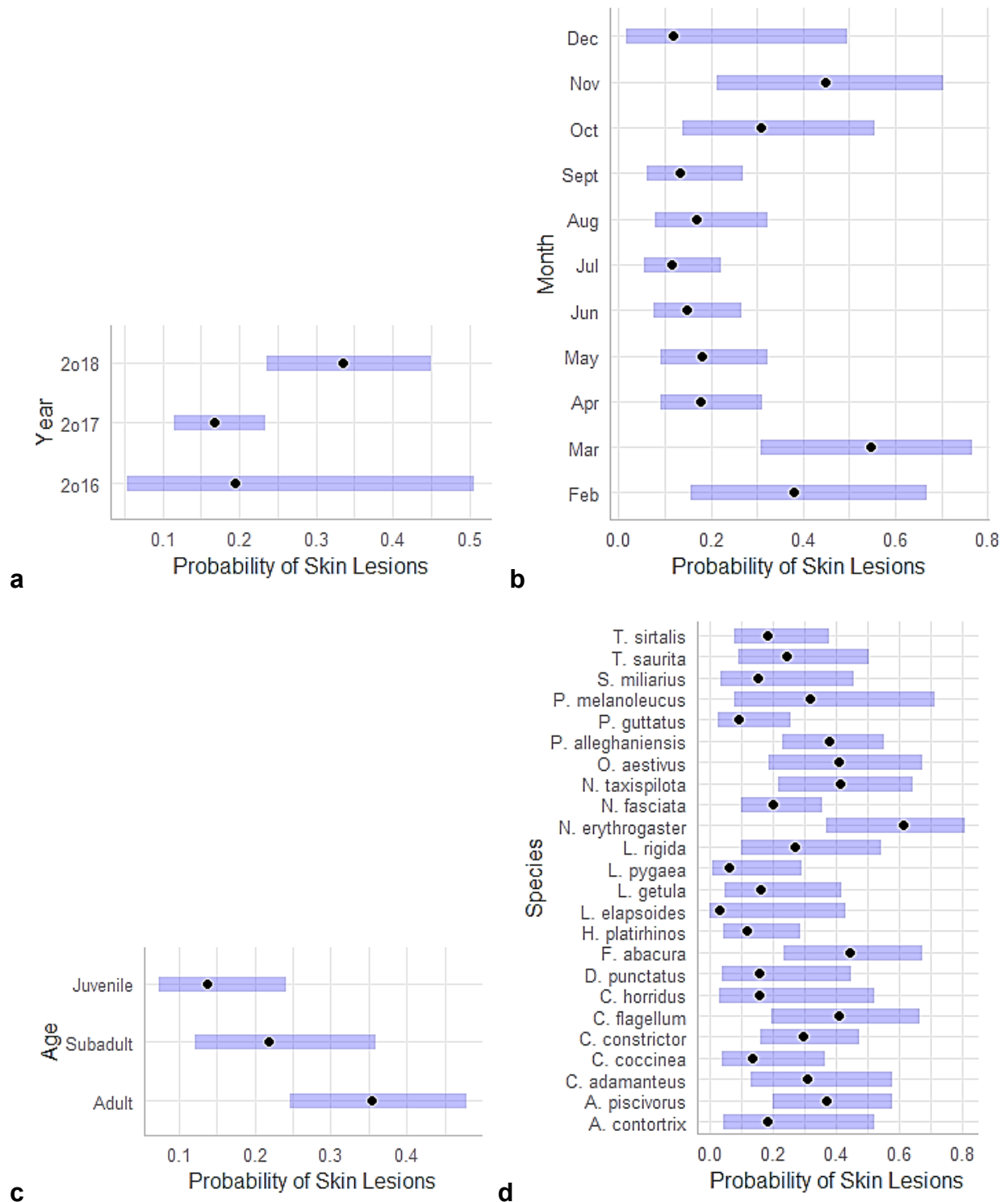
Month	Number sampled	2016	2017	2018
January	5	0	5	0
February	18	0	9	9
March	33	0	25	8
April	107	0	44	63
May	96	0	43	53
June	108	0	62	46
July	113	0	54	59
August	72	0	56	16
September	54	13	41	0
October	34	3	31	0
November	28	3	25	0
December	11	4	7	0
TOTAL:	679	23	402	254

Supplementary Table S13: Contingency table showing number of snakes sampled by age class and ophidiomycosis category in Georgia, USA from 2016 to 2018.

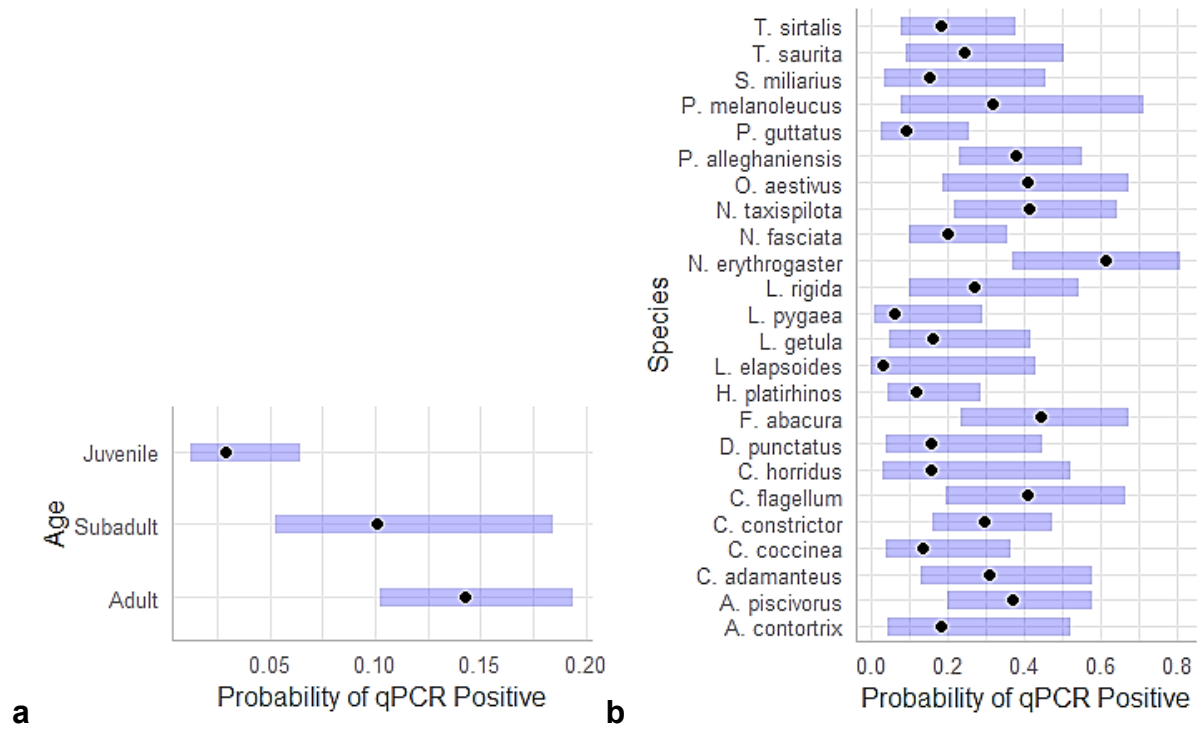
Ophidiomycosis Category					
Age Class	Number sampled	Negative	<i>Ophidiomyces</i> present	Possible ophidiomycosis	Apparent ophidiomycosis
Juvenile	150	125	1	20	4
Subadult	101	74	4	16	7
Adult	428	273	15	81	59
TOTAL:	679	472	20	117	70

Supplementary Table S14: Contingency table showing number of snakes sampled by species and ophidiomycosis category in Georgia, USA from 2016 to 2018.

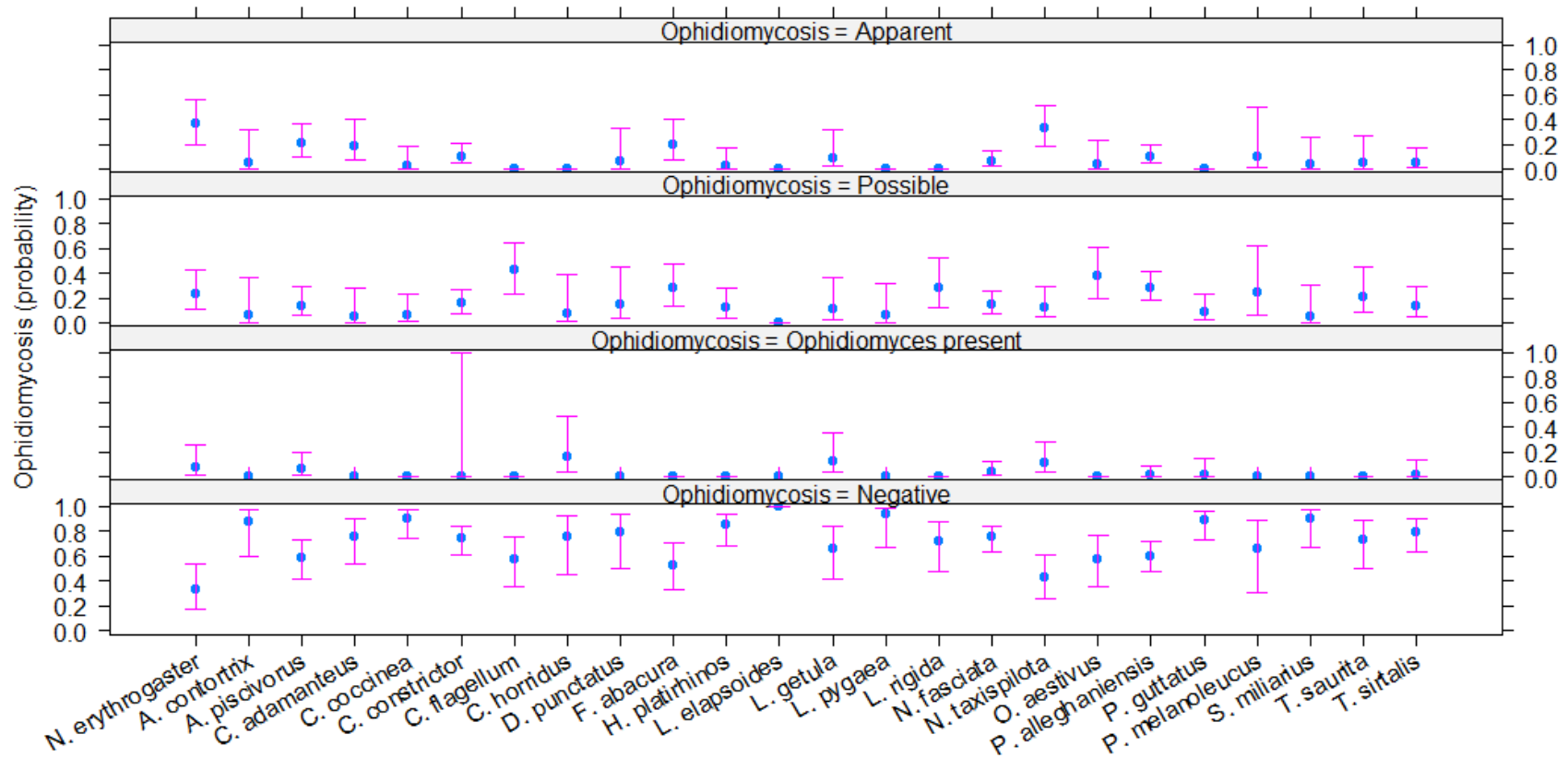
Species	Number sampled	Ophidiomycosis category			
		Negative	<i>Ophidiomyces</i> present	Possible ophidiomycosis	Apparent ophidiomycosis
<i>Agkistrodon contortrix</i>	13	11	0	1	1
<i>Agkistrodon piscivorus</i>	39	23	3	5	8
<i>Cemophora coccinea</i>	28	25	0	2	1
<i>Coluber constrictor</i>	57	40	0	10	7
<i>Coluber flagellum</i>	25	15	0	10	0
<i>Crotalus adamanteus</i>	25	18	0	2	6
<i>Crotalus horridus</i>	15	12	2	1	0
<i>Diadophis punctatus</i>	11	8	0	2	1
<i>Farancia abacura</i>	27	14	0	10	4
<i>Farancia erytrogramma</i>	4	2	0	1	1
<i>Haldea striatula</i>	2	1	0	1	0
<i>Heterodon platirhinos</i>	35	31	0	4	1
<i>Heterodon simus</i>	3	2	0	1	0
<i>Lampropeltis elapsoides</i>	8	8	0	0	0
<i>Lampropeltis getula</i>	16	9	3	2	2
<i>Liodytes alleni</i>	4	3	0	1	0
<i>Liodytes pygaea</i>	15	13	0	1	0
<i>Liodytes rigida</i>	20	11	0	4	1
<i>Micrurus fulvius</i>	4	4	0	0	0
<i>Nerodia erythrogaster</i>	27	9	2	6	10
<i>Nerodia fasciata</i>	69	51	3	9	4
<i>Nerodia floridana</i>	2	1	0	1	0
<i>Nerodia taxispilota</i>	33	14	4	4	11
<i>Opheodrys aestivus</i>	19	10	0	8	1
<i>Pantherophis alleghaniensis</i>	61	36	1	21	7
<i>Pantherophis guttatus</i>	32	28	1	3	0
<i>Pituophis melanoleucus</i>	9	5	0	3	1
<i>Sistrurus miliarius</i>	16	14	0	1	1
<i>Storeria dekayi</i>	2	2	0	0	0
<i>Storeria occipitomaculata</i>	2	2	0	0	0
<i>Thamnophis sauritus</i>	19	9	0	4	1
<i>Thamnophis sirtalis</i>	36	27	1	5	2
<i>Virginia valeriae</i>	1	1	0	0	0
TOTAL:	679	472	20	117	70



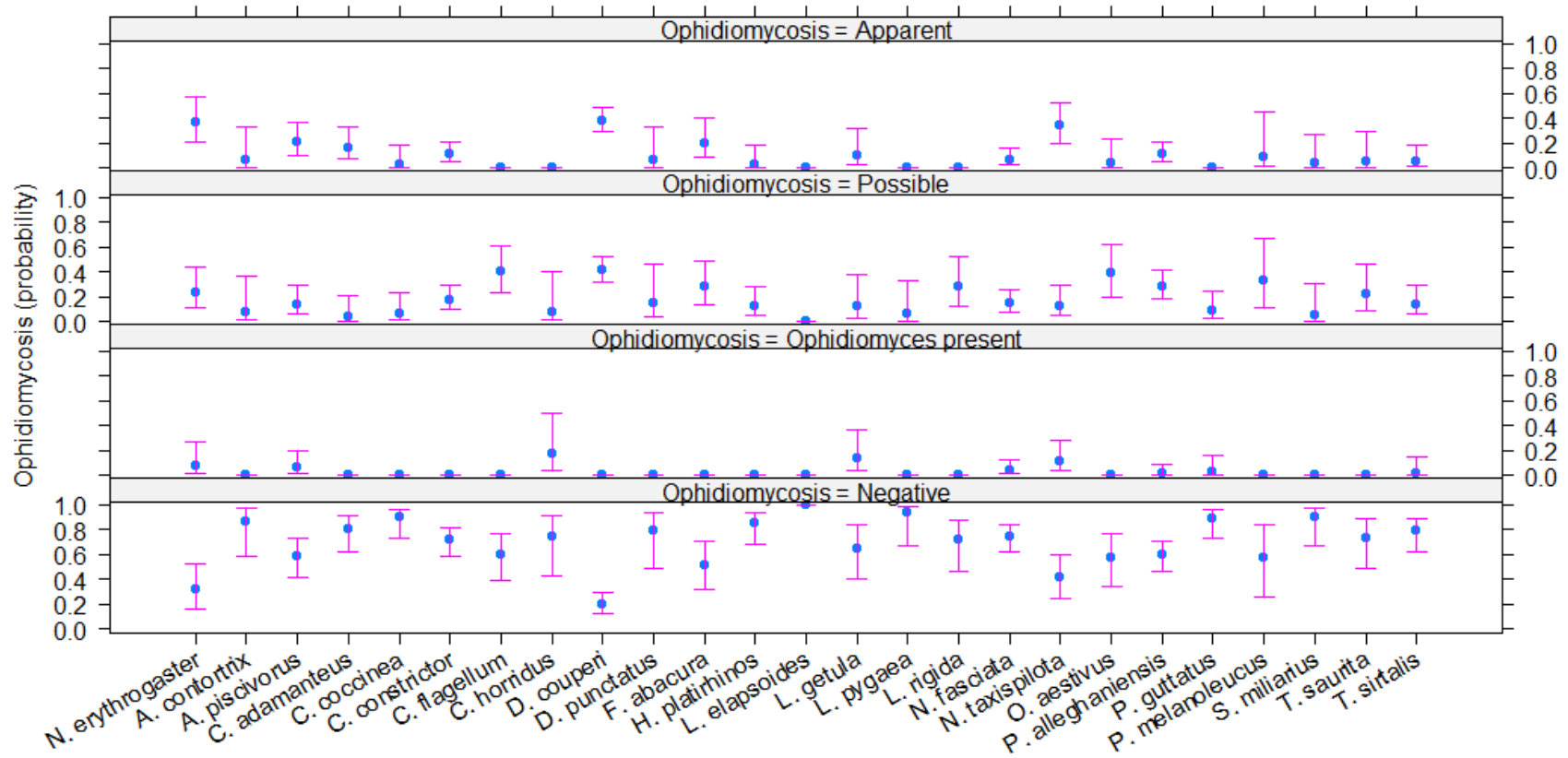
Supplementary Figure S1. Effect size plots (marginal mean values with 95% confidence intervals) estimated from a generalized linear model predicting the probability of skin lesion presence using the additive effects of year (a), month (b), age class (c), and species (d).



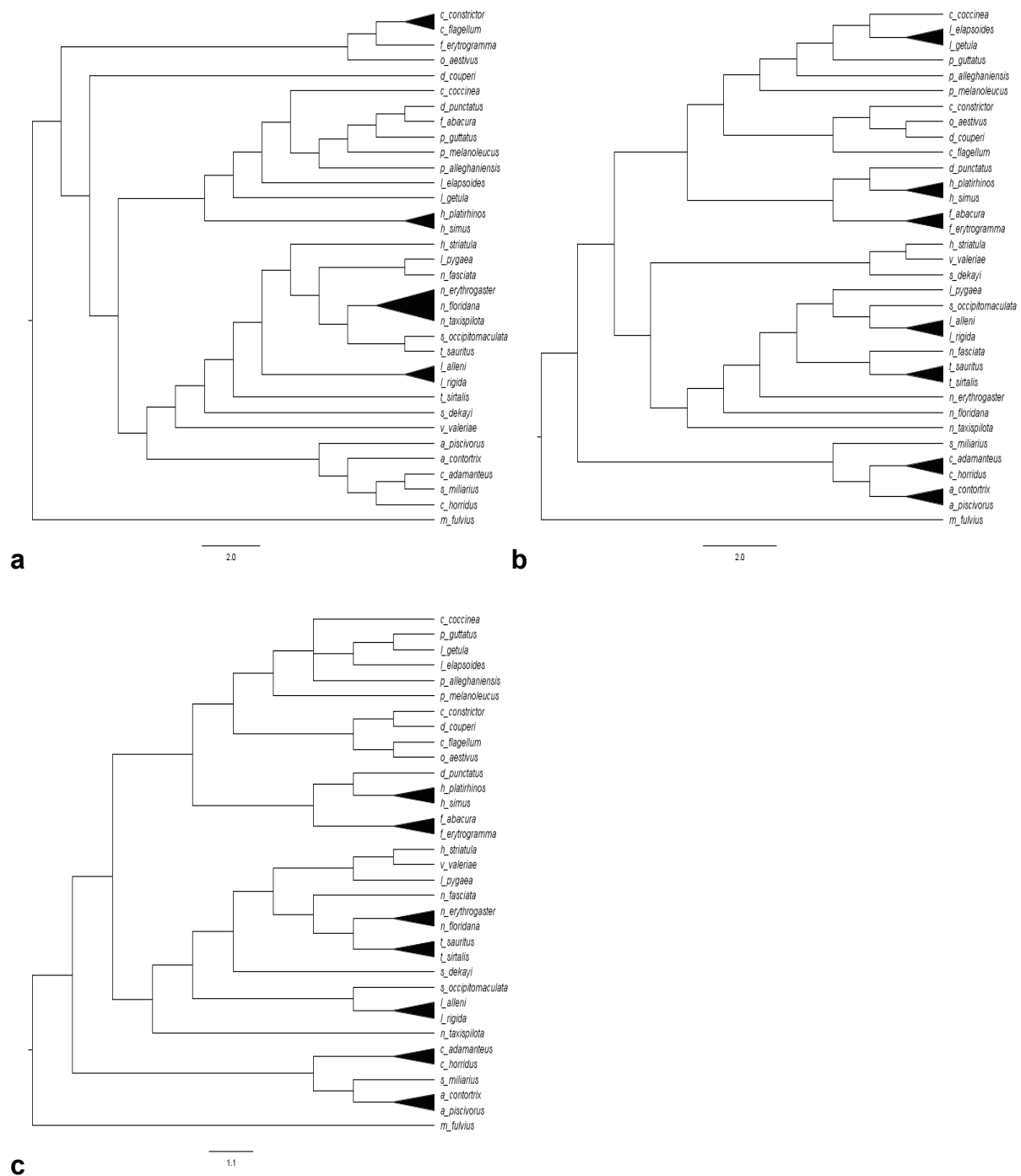
Supplementary Figure S2. Effect size plots (marginal mean values with 95% confidence intervals) estimated from a generalized linear model predicting the probability of testing qPCR positive for *Ophidiomyces ophiodiicola* using the additive effects of age class (a) and species (b).



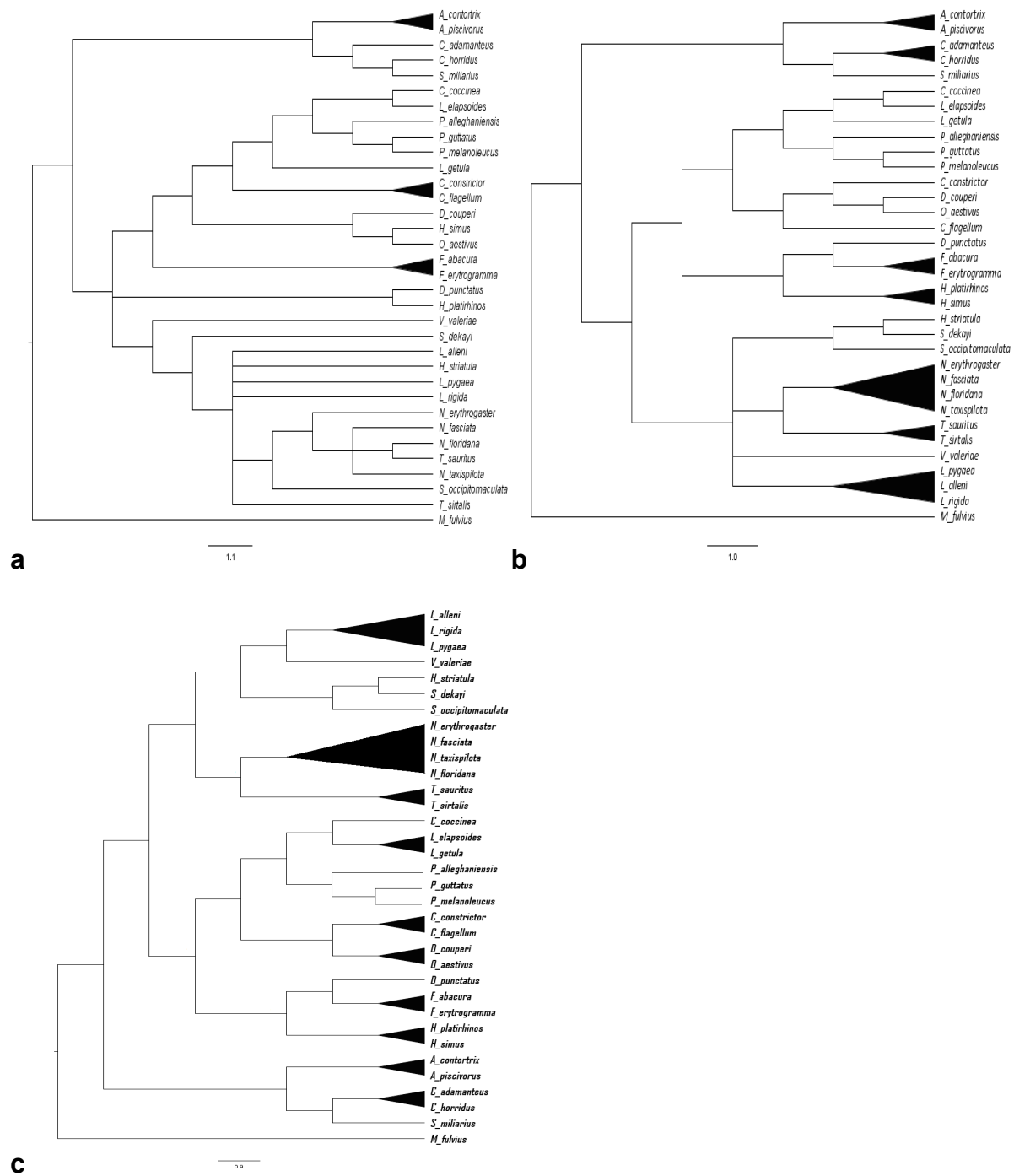
Supplementary Figure S3. Effect size plots (marginal mean values with 95% confidence intervals) estimated from a multinomial logistic regression model predicting ophidiomycosis category using the additive effects of age class and species. Eastern indigo snakes (*Drymarchon couperi*) are excluded.



Supplementary Figure S4. Effect size plots (marginal mean values with 95% confidence intervals) estimated from a multinomial logistic regression model predicting ophidiomyces category using the additive effects of age class and species. Eastern indigo snakes (*Drymarchon couperi*) are included.



Supplemental Figure S5. Phylogenetic reconstruction from protein sequences using different algorithms: **(a)** Least squares distance algorithm; **(b)** Maximum parsimony algorithm; **(c)** Maximum likelihood algorithm. Triangles indicate species of the same genus grouped together on adjacent branches of the tree.



Supplemental Figure S6. Phylogenetic reconstruction from cDNA sequences using different algorithms: **(a)** Least squares distance algorithm; **(b)** Maximum parsimony algorithm; **(c)** Maximum likelihood algorithm. Triangles indicate species of the same genus grouped together on adjacent branches of the tree.