

1 **Supplementary Table S1** Exemplary overview of acute toxicity (96-h LC₅₀) of H₂S on juveniles and adults of some widespread
 2 North American fishes that often co-occur with poeciliid fishes in non-sulfidic habitats. Information extracted from the United
 3 States Environmental Protection Agency ECOTOX database (<http://cfpub.epa.gov/ecotox/>).
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Common name	Species name	Family	96-h LC ₅₀	Reference
Fathead minnow	<i>Pimephales promelas</i>	Cyprinidae	<1 μM/L (24 μg/L) at 20°C 1-2 μM/L (39-43 μg/L) at 16-18°C	Smith et al. 1976 Smith & Oseid 1970
Walleye	<i>Sander vitreus</i>	Percidae	<1 μM/L (18-20 μg/L) at 16-18°C	Smith & Oseid 1970
Bluegill sunfish	<i>Lepomis macrochirus</i>	Centrarchidae	ca. 1 μM/L (20-32 μg/L) at 16-18°C	Smith & Oseid 1970
Brook trout	<i>Salvelinus fontinalis</i>	Salmonidae	ca. 1 μM/L (20-30 μg/L)	Smith & Oseid 1974

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 7 **Supplementary Table S2** Overview of sample sites and locations used for the life-history analyses. H₂S-concentrations in
 8 nontoxic habitats range from -1.6 to 1.4 μM, H₂S-concentrations for sulfidic habitats are provided as mean ± SD.

Species	Location	H ₂ S [μM]	N	Latitude	Longitude	Source
<i>Gambusia affinis</i>	Trib. Washita River	-	29	35° 0'7.94"N	98°41'16.03"W	Collected by authors, July 2009
	Travertine Creek	-	44	34°30'14.90"N	96°58'16.68"W	Collected by authors, July 2009
	Vendome Well	+ [40.5 ± 30.6]	31	34°30'21.28"N	96°58'19.51"W	Collected by authors, July 2009
<i>Gambusia holbrooki</i>	Gleason Park	-	62	28° 8'40.03"N	80°35'50.39"W	Collected by authors, August 2011
	Green Springs	+ [49.4 ± 3.2]	33	28°51'47.06"N	81°14'54.83"W	Collected by authors, August 2011
	Lake Monroe	-	29	28°51'44.39"N	81°15'9.91"W	Collected by authors, August 2011

<i>Gambusia hubbsi</i>	Archie's blue hole	+	36	24°54'4.56"N	77°56'10.77"W	Collected by authors, August 2002
	London Pond	-	26	24°54'35.99"N	77°59'4.00"W	Collected by authors, August 2002
	Thompson/Scott	-	11	24°54'32.03"N	77°56'8.99"W	Collected by authors, August 2002
<i>Gambusia eurystoma</i>	Baños del Azufre	+ [190.4±119.7]	44	17°33'9.20"N	92°59'51.45"W	Collected by authors, January 2009
<i>Gambusia sexradiata</i>	Mogote del Puyacatengo	+ [7.5 ± 3.2]	16	17°34'55.87"N	92°53'59.51"W	Collected by authors, January 2009
	Río Teapao	-	34	17°33'24.90"N	92°57'3.54"W	Collected by authors, January 2009
<i>Poecilia latipinna</i>	Carrabelle	-	19	29°47'52.32"N	84°44'40.68"W	Collected by authors, June 2008
	Panacea Mineral Springs	+ [7.9 ± 2.6]	13	30° 2'4.14"N	84°23'23.34"W	Collected by authors, June 2008
	Sawgrass Lake Park	-	16	27°50'56.70"N	82°40'4.56"W	Collected by authors, June 2008
<i>Poecilia mexicana</i>	Arroyo Bonita	-	33	17°25'37.42"N	92°45'6.98"W	Collected by authors, January 2008
	Arroyo Rosita	-	37	17°29'6.40"N	93° 6'12.89"W	Collected by authors, September 2010

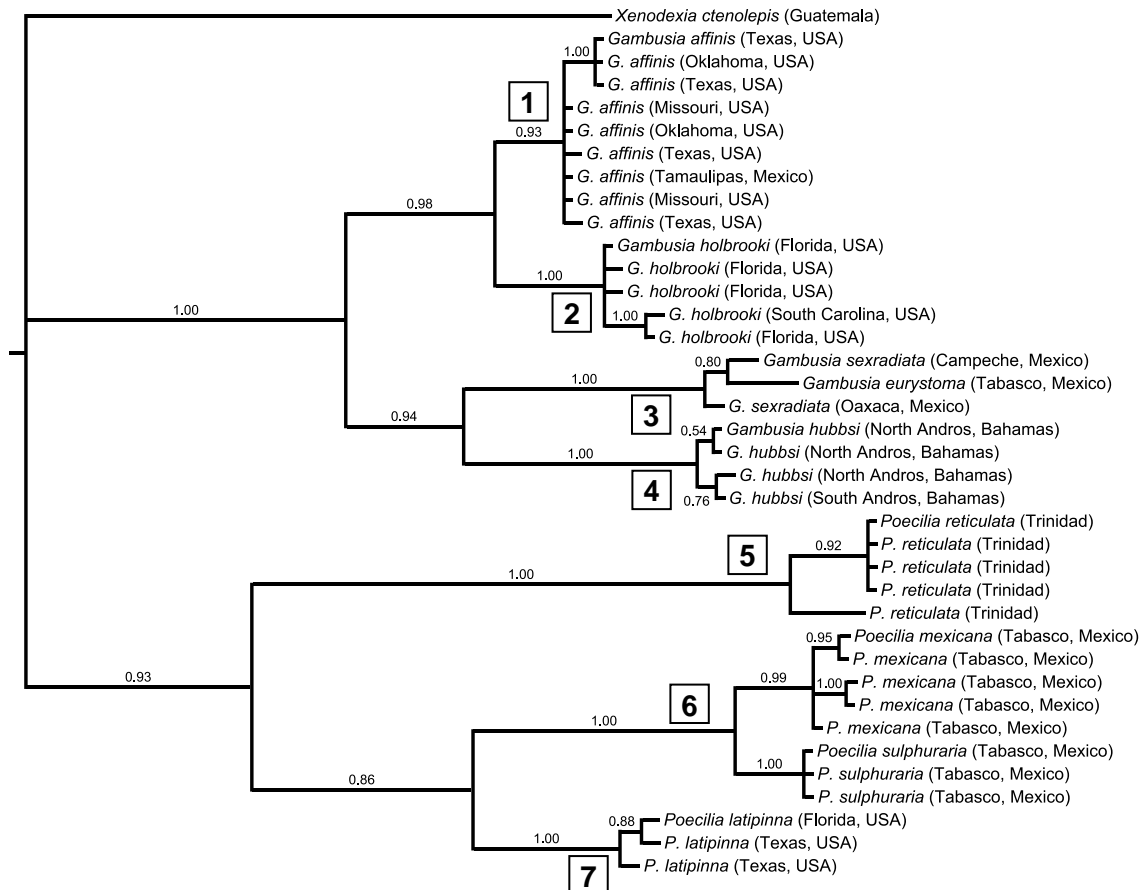
	El Azufre	+ [23.7±18.2]	23	17°26'32.10"N	92°46'28.09"W	Collected by authors, January 2008
	Río Amatan	-	23	17°25'59.92"N	92°47'34.55"W	Collected by authors, June 2007
<i>Poecilia sulphuraria</i>	La Gloria	+ [154.8±54.5]	27	17°31'55.24"N	93° 0'54.47"W	Collected by authors, September 2010
<i>Poecilia reticulata</i>	Caño Volcan	-	16	8°59'15.00"N	69°52'59.88"W	TNHC 14971*
	Pozo Azufre	+ [n/a]	8	10°16'59.88"N	63° 7'0.12"W	TNHC 14968*, 14969*
	Trib. Caño Volcan	-	13	8°59'15.04"N	69°53'29.95"W	TNHC 14982*

9 * for details please refer to Winemiller *et al.* (1990)

Supplementary Table S3 GenBank accession numbers and locality information for each sample used in phylogenetic examination of cytochrome *b* gene sequences.

Species	Locality	GenBank Acc. #
<i>Xenodexia ctenolepis</i>	Guatemala	EF017557
<i>Gambusia affinis</i>	Brazos County, Texas	HM443906
<i>Gambusia affinis</i>	Shannon County, Missouri	HM443903
<i>Gambusia affinis</i>	Marshall County, Oklahoma	HM443907
<i>Gambusia affinis</i>	Cleveland County, Oklahoma	HM443905
<i>Gambusia affinis</i>	Maverick County, Texas	HM443905
<i>Gambusia affinis</i>	Tamaulipas, Mexico	HM443902
<i>Gambusia affinis</i>	Merrimack River, Missouri	EF017514
<i>Gambusia affinis</i>	Texas	U18107
<i>Gambusia affinis</i>	Menard County, Texas	DQ075681
<i>Gambusia holbrooki</i>	Miami-Dade County, Florida	HM443916
<i>Gambusia holbrooki</i>	Manatee County, Florida	HM443917
<i>Gambusia holbrooki</i>	Monroe County, Florida	HM443915
<i>Gambusia holbrooki</i>	Richland/Lexington Counties, South Carolina	HM443918
<i>Gambusia holbrooki</i>	Florida	U18210
<i>Gambusia sexradiata</i>	Campeche, Mexico	KF704051
<i>Gambusia sexradiata</i>	Oaxaca, Mexico	U18224
<i>Gambusia eurystoma</i>	Tabasco, Mexico	U18206
<i>Gambusia hubbsi</i>	North Andros Island, Bahamas	KF704052
<i>Gambusia hubbsi</i>	North Andros Island, Bahamas	KF704053
<i>Gambusia hubbsi</i>	North Andros Island, Bahamas	KF704054
<i>Gambusia hubbsi</i>	South Andros Island, Bahamas	KF704055
<i>Poecilia reticulata</i>	Trinidad	EF017536
<i>Poecilia reticulata</i>	Trinidad	GU179192
<i>Poecilia reticulata</i>	Trinidad	GQ855720
<i>Poecilia reticulata</i>	Trinidad	GQ855736
<i>Poecilia reticulata</i>	Trinidad	GQ855710
<i>Poecilia mexicana</i>	Chiapas, Mexico	KF704058
<i>Poecilia mexicana</i>	Tabasco, Mexico	KF704056
<i>Poecilia mexicana</i>	Tabasco, Mexico	KF704057
<i>Poecilia mexicana</i>	Tabasco, Mexico	KF704059
<i>Poecilia mexicana</i>	Tabasco, Mexico	KF704060
<i>Poecilia sulphuraria</i>	Tabasco, Mexico	KF704061
<i>Poecilia sulphuraria</i>	Tabasco, Mexico	KF704062
<i>Poecilia sulphuraria</i>	Chiapas, Mexico	KF704063
<i>Poecilia latipinna</i>	Wakulla County, Florida	HQ677867
<i>Poecilia latipinna</i>	Cameron County, Texas	HQ677868
<i>Poecilia latipinna</i>	Cameron County, Texas	FJ446153

Supplementary Figure S1 Bayesian phylogenetic hypothesis for the 38 samples using the mtDNA *cyt b* gene fragment. Topology represents the 50% majority-rule consensus tree, and numbers indicate Bayesian posterior probability for each node. Bold numbers denote the seven major clades revealed by this analysis and used in analyses described in the text.



References

1. Smith, L.L.J., Oseid, D.M. & Olson, L.E. (1975) Acute and chronic toxicity of hydrogen sulfide to the fathead minnow, *Pimephales promelas*. *Environ. Sci. Technol.*, **10**, 565–568.
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