



***Angiostoma lamotheargumedoi* n. sp. (Nematoda: Angiostomatidae) from the intestine of *Pseudoeurycea mixteca* (Caudata: Plethodontidae) in central Mexico**

***Angiostoma lamotheargumedoi* n. sp. (Nematoda: Angiostomatidae) del intestino de *Pseudoeurycea mixteca* (Caudata: Plethodontidae) en la región central de México**

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Abstract. A new species of *Angiostoma* (Angiostomatidae) is described from the intestine of the plethodontid salamander, *Pseudoeurycea mixteca* from Puebla State, in central Mexico. The new species closely resembles *Angiostoma limancis*, *Angiostoma kimmeriensis*, *Angiostoma spiridonovi*, *Angiostoma stammeri*, and *Angiostoma carettae*, because they all possess 8 pairs of pedunculate papillae in the caudal region; however, the new species can be distinguished by the unique arrangement of papillae, with 1 pre-cloacal pair, and 7 post-cloacal pairs. Among the 12 congeneric species of *Angiostoma* described so far, 2 have been described as parasites of salamanders, *Angiostoma plethodontis* from *Plethodon cinereus* and *Plethodon richmondi* in Virginia, USA, and *Angiostoma onychodactyla* from *Onychodactylus japonicus* in Japan. *Angiostoma lamotheargumedoi* n. sp. is readily distinguished from these 2 species by the presence of lateral alae and by the number of pre-cloacal papillae.

Key words: *Angiostoma lamotheargumedoi* n. sp., Angiostomatidae, *Pseudoeurycea mixteca*, Plethodontidae, Puebla State, Mexico.

Resumen. En este trabajo se describe una especie nueva del género *Angiostoma* (Angiostomatidae) como parásito del intestino de la salamandra plethodóntida *Pseudoeurycea mixteca* en el estado de Puebla, en el centro de México. La nueva especie es morfológicamente similar a *Angiostoma limancis*, *Angiostoma kimmeriensis*, *Angiostoma spiridonovi*, *Angiostoma stammeri* y *Angiostoma carettae*, porque todas ellas poseen 8 pares de papilas pedunculadas en la región caudal; sin embargo, se puede distinguir de éstas por el arreglo característico de dichas papilas: 1 par en posición precloacal y 7 pares postcloacales. Dos de las 12 especies congénicas de *Angiostoma* fueron descritas de salamandras, *Angiostoma plethodontis* de *Plethodon cinereus* y *Plethodon richmondi* en Virginia, EUA y *Angiostoma onychodactyla* de *Onychodactylus japonicus* en Japón. *Angiostoma lamotheargumedoi* n. sp. se distingue fácilmente de estas 2 especies por la presencia de alas laterales y por el número de papilas precloacales.

Palabras clave: *Angiostoma lamotheargumedoi* n. sp., Angiostomatidae, *Pseudoeurycea mixteca*, Plethodontidae, Puebla, México.

Introduction

Among the members of the supergenus *Bolitoglossa* Dumeril, Bibron and Dumeril, 1854, the most specious salamander group in the neotropics (Wiens et al., 2007), the genus *Pseudoeurycea* Taylor, 1944, includes about 40 species that mainly occur in high elevation forests ranging from northern Mexico into western Guatemala (Parra-Olea, 2002). Many species show localized distributions, some of them restricted to a particular locality, or mountain range;

consequently, salamander endemism is very high in the country (Parra-Olea et al., 2004, 2005; Canseco-Márquez and Gutierrez-Mayén, 2005). *Pseudoeurycea mixteca* Canseco-Márquez and Gutierrez-Mayén, 2005 was thought to be restricted to western Oaxaca, in high elevation pine forests, in the Sierra Mixteca Oaxacense but it was recently reported from the northern most part of the Mixteca in the State of Puebla (Windfield-Pérez et al., 2007). Specimens of *P. mixteca* were studied for helminths as a part of an ongoing inventory of the helminth parasites of Neotropical plethodontid salamanders, and a new species of nematode of the genus *Angiostoma* Dujardin, 1845 was found.

Members of *Angiostoma* are mainly parasites of terrestrial gastropods, with 9 species distributed in Western Europe and Australia; however, 2 species have been found in salamanders and, more recently, 1 species was described from the lungs of the loggerhead sea turtle, *Caretta caretta* (Linnaeus, 1758) (Bursey and Manire, 2006). *Angiostoma plethodontis* Chitwood, 1933, was described from the northern redback salamander, *Plethodon cinereus* Green in Virginia, USA (Chitwood, 1933), and *Angiostoma onychodactyla* Bursey and Goldberg, 2000, was described from the Japanese clawed salamander, *Onychodactylus japonicus* (Houttuyn) in Honshu, Japan (Bursey and Goldberg, 2000). In this paper, a new species of *Angiostoma* from the intestine of *P. mixteca* in central Mexico is described.

Materials and methods

Fourteen plethodontid salamanders (*P. mixteca*) were collected by hand in a sinkhole near the locality of Tepanco de López, Puebla State, in December, 2004, and July, 2006 (Fig. 1). After capture, salamanders were killed with an overdose of anesthetic (sodium pentobarbital), and dissected. Nematodes were recovered by opening the intestine and rectum with small blunt-nosed scissors and searching the mucosal side with a stereoscope. Nematodes were placed in 8.5% saline, fixed with glacial acetic acid, and stored in 70% ethanol. Specimens were cleared for study with lactophenol. Drawings and measurements were made with a Zeiss microscope equipped with a drawing tube. For scanning electron microscopy (SEM) study, nematodes were dehydrated in series of gradual ethanol and critical point dried with carbon dioxide. Specimens were coated with gold and examined in a Hitachi S-2460N scanning electron microscope at 15kV. Measurements are given in micrometers (μm) unless otherwise indicated, and presented as the range, with the mean, standard deviation, and sample size in brackets, followed by the measurements of the holotype and allotype in parentheses. The specimens were deposited in the Colección Nacional de Helmintos (CNHE), Instituto de Biología, Universidad Nacional Autónoma de México (UNAM). Hosts were deposited at the Colección Nacional de Anfibios y Reptiles (CNAR), Instituto de Biología, Universidad Nacional Autónoma de México (UNAM) with the accession numbers: IBH 14350, 18701-18716. For comparison, specimens deposited at the U.S. National Parasite Collection (USNPC) were studied as follow: *A. plethodontis* ex *Triturus vulgaris vulgaris* (Linnaeus, 1758) (065662), *A. onychodactyla* ex *O. japonicus* (088647), *Angiostoma aspersae* Morand, 1986 ex *Mertensiella luschni* (Steindachner, 1891) (094464),

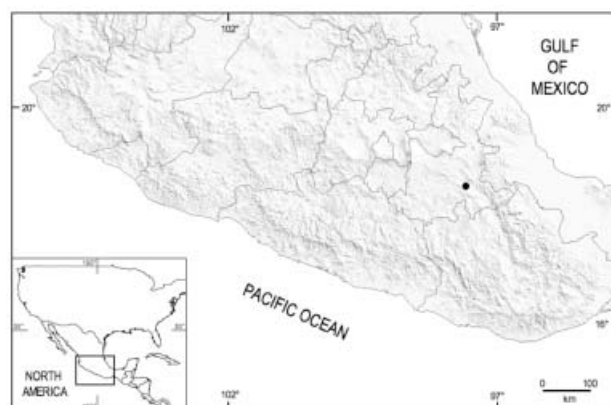


Figure 1. Map of Mexico showing the locality where the plethodontid salamanders were collected in Puebla State.

and *Angiostoma carettae* Bursey and Manire, 2006 ex *C. caretta* (095696).

Description

Angiostoma lamothearguedoi n. sp. (Figs. 2A-F, 3A-B)

Small nematodes with transparent body. Two pairs of cuticular lateral alae present, both starting approximately at the level of the esophageal isthmus and extending posteriorly to the level of the bursa in males, and to the level of the tail in females. Oral opening wide, with 3 lips and 2 amphids. Thick-walled buccal cavity. Esophagus with isthmus and posterior glandular bulb. Male with 1 pair of well-developed caudal alae supported by 8 pedunculated papillae; spicules paired, similar, proximal end thickened, pointed distal end; gubernaculum present. Females with pre-equatorial vulva, near mid-body; vagina short; amphidelphic; ovaries flexed; eggs were not observed.

Male: Based in 12 mature specimens. Length 1.19–2.29 mm ($1.69 \text{ mm} \pm 0.33$, $n = 11$) (1.75 mm); maximum width 50–70 (60 ± 6 , $n = 11$) (60). Buccal cavity 6–15 (14 ± 3 , $n = 11$) (15) long. Esophagus 150–220 (190 ± 21 , $n = 10$) (200) long, consisting of corpus 75–140 (112 ± 18 , $n = 10$) (111) long, isthmus 30–70 (50 ± 12 , $n = 10$) (45) long, and bulb 20–35 (28 ± 5 , $n = 10$) (30) long, 18–21 (20 ± 2 , $n = 10$) (21) wide. Distance from anterior end to nerve ring (60) and to excretory pore (162). Spicules equal, 50–80 (68 ± 9 , $n = 11$) (55), well sclerotized and curved. Gubernaculum well-sclerotized, 20–30 (27 ± 4 , $n = 9$) (28) long. Two caudal alae well-developed supported by 8 pairs of pedunculate papillae; 1 pair precloacal, 7 pairs postcloacal arranged in 3 groups, 2 pairs close to cloaca, 3 pairs midway between cloaca and tail, and 2 pairs close to tail. Tail extending beyond bursa.

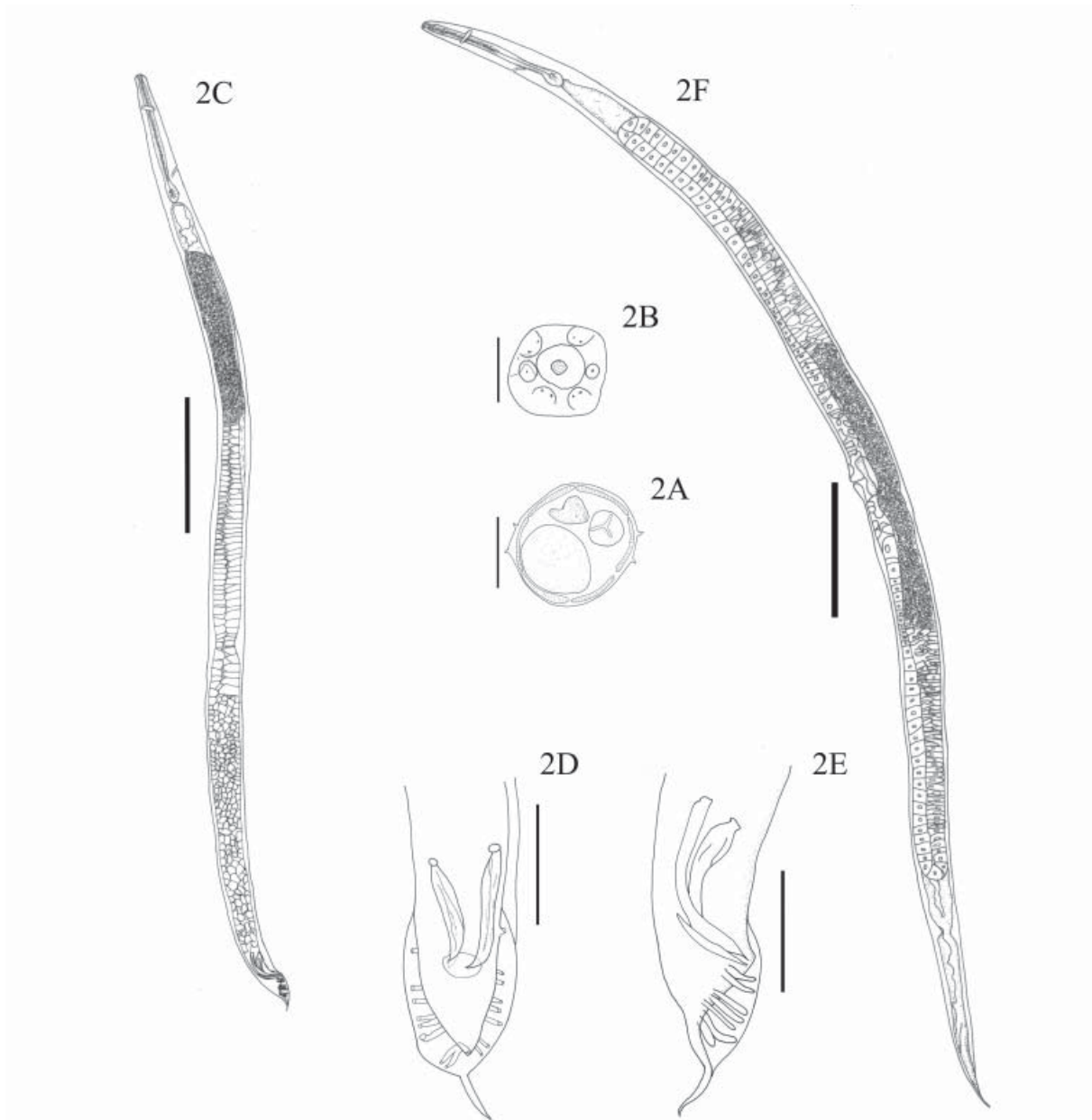


Figure 2. *Angiostoma lamotheargumedi* n. sp. 2A, cross section at midbody of a male showing 2 pairs of lateral alae (scale bar: 0.05 mm). 2B, in face view of male showing lips and papillae (scale bar: 0.01 mm). 2C, male, entire, lateral view (scale bar: 0.25 mm). 2D, posterior end of male, ventral view showing caudal alae, pedunculated papillae and spicules (scale bar: 0.05 mm). 2E, posterior end of male, lateral view. 2F, female, entire, lateral view (scale bar: 0.25 mm).

Female: From 15 mature specimens. Length 1.32-2.25 mm ($1.9 \text{ mm} \pm 0.35$, $n = 14$) (2.14 mm), maximum width at level of vulva 40-75 (59 ± 11 , $n = 14$) (72). Buccal cavity 9-18 (15 ± 2 , $n = 14$) (15), long. Esophagus 156-219 (192 ± 19 , $n = 13$) (210) long, consisting of corpus 66-135 (115 ± 17 , $n = 11$) (126) long, isthmus 30-63 (47

± 13 , $n = 11$) (54) long, and bulb 24-33 (29 ± 3 , $n = 11$) (30) long, 18-30 (22 ± 3 , $n = 11$) (21) wide. Distance from anterior end to nerve ring (81) and to excretory pore (180). Vulva 580-1000 (848 ± 135 , $n = 12$) (984) from anterior end. Tail conical 36-123 (75 ± 23 , $n = 14$) (63) long. Eggs not observed in uterus.

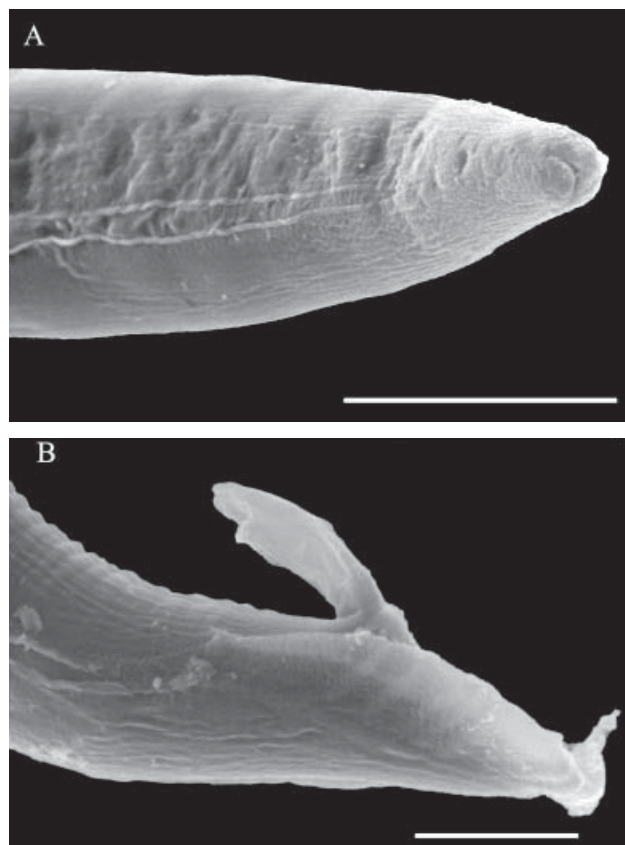


Figure 3. Scanning electron micrographs of *Angiostoma lamotheargumedei* n. sp. 3A, anterior end of male showing the 1 pair of lateral alae (scale bar: 0.04 mm). 3B, posterior end of male, lateral view, showing caudal alae with pedunculated papillae and spicules (scale bar: 0.02 mm) and the posterior end of lateral alae.

Taxonomic Summary

Type-host: *Pseudoeurycea mixteca* Canseco-Márquez and Gutiérrez-Mayén, 2005.

Site: intestine.

Type-locality: a sinkhole near the locality of Tepanco de López, Puebla State (19°20'20"N, 98°43'14"W, altitude: 3230 m.).

Prevalence and mean abundance: 6/14, 42.8% (7.2 worms per analyzed host).

Type-specimens: holotype (5914), allotype (5915), paratypes (5916-5917), vouchers (5918-5919), deposited in the Colección Nacional de Helminths, México D.F., Mexico (CNHE).

Etymology: the species is named after Dr. Rafael Lamothe Argumedo in recognition of his outstanding contribution to Mexican parasitology.

Remarks

So far, 12 species of *Angiostoma* have been described; 9 as parasites of terrestrial gastropods, 1 in a marine turtle, and 2 in salamanders (Table 1). *Angiostoma lamotheargumedei* n. sp. closely resembles *Angiostoma limancis* Dujardin, 1845, *A. kimmeriensis* Korol and Spiridonov, 1991, *A. spiridonovi* Morand, 1992, *A. stammeri* Mengert, 1953, and *A. carettae*, because they all possess 8 pairs of pedunculate papillae in the caudal region; however, the new species can be distinguished by the unique arrangement of papillae, with 1 pre-cloacal pair, and 7 post-cloacal pairs. In addition, the aforementioned species lack lateral alae, and the new species is characterized by having 2 pairs of cuticular lateral alae.

Two species of *Angiostoma* have been described as parasites of salamanders, and 1 of them in particular in plethodontids. *Angiostoma plethodontis* was described by Chitwood (1933) as a parasite of *P. cinereus* in Virginia, USA, and more recently was recorded from *Plethodon richmondi* Nettin and Mittleman, 1938, in the same locality by Emery and Joy (2000). The second species is *A. onychodactyla* from the hynobiid salamander *O. japonicus* in Japan. The new species is readily distinguished from the 2 congeneric species found in salamanders by the presence of lateral alae and by the number of pre-cloacal papillae. *Angiostoma plethodontis* and *A. onychodactyla* lack lateral alae and there are 2 pairs of pre-cloacal papillae or no papillae, respectively, whereas in the new species only 1 pair of pre-cloacal papillae is present.

The current inventory of the helminth parasites of Neotropical plethodontid salamanders is still in initial stages, with the nematode *Cosmocerca acanthurum* Falcón-Ordaz, Windfield-Pérez, Mendoza-Garfias, Parra-Olea and Pérez-Ponce de León, 2007 being the first species described (Falcón-Ordaz et al., 2007). These authors predicted that the helminth fauna of neotropical salamanders would be found to be species-poor, with low abundance values, and mainly be dominated by nematodes with direct or indirect life-cycles. The finding presented herein provide additional support for that prediction since only 1 species of nematode was found as a parasite of *P. mixteca*. The microhabitat where the host was reported is quite unusual for a plethodontid salamander, since they were found in a sinkhole at the border between oak forest and xerophile vegetation. Adult organisms were found in the sinkhole at about 80 meters below surface level under stones in humid sand or burrowed about 30 cm beneath the sinkhole surface (Windfield-Pérez et al., 2007). This fact poses interesting questions regarding the infection mechanism of this species. Most probably, the new species could have been a result of a host-switching event from

Table 1. List of species of *Angiostoma* with their main diagnostic traits

	Host	Lateral alae	Pre-cloacal papillae (pairs)	Adanal papillae (pairs)	Post-cloacal papillae (pairs)	Spicules μm	Gubernaculum μm
<i>A. limancis</i> Dujardin, 1845	<i>Arion ater</i>	Absent	3		6	110	40
<i>A. kimmeriensis</i> Korol and Spiridonov, 1991	<i>Oxychilus deilus</i>	Absent	3		5	125	50
<i>A. schizoglossae</i> Morand and Baker, 1995	<i>Schizoglossa novoseelandica</i>	Absent	3	1	6	80	25
<i>A. spiridonovi</i> Morand, 1992	<i>Limas flavus</i>	Absent	2	1	5	82	35
<i>A. stammeri</i> Mengert, 1953	<i>Limas cinereoniger</i> and <i>L. maximus</i>	Absent	2	1	5	101-125	65-70
<i>A. aspersae</i> Morand, 1986	<i>Helix aspersa</i>	Present	4 (+ 1)		7	75	35
<i>A. dentifera</i> Mengert, 1953	<i>Limas cinereoniger</i>	Present	5		4	55	30
<i>A. asamati</i> Spiridonov, 1985	<i>Turcomilax ferganus</i>	Present	2		4	136	47
<i>A. coloaense</i> Van Luc, Spiridonov and Wilson, 2005	<i>Cyclophorus</i> sp.	Present	2		7	138	58
<i>A. carettae</i> Bursey and Manire, 2006	<i>Caretta caretta</i>	Absent	3		5	41	18
<i>A. plethodontis</i> Chitwood, 1933	<i>Plethodon cinereus</i>	Absent	2		7	60	25
<i>A. onychodactyla</i> Bursey and Goldberg, 2000	<i>Onychodactylus japonicus</i>	Absent	0		8	128	44
<i>A. lamotheargumedoi</i> n. sp.	<i>Pseudoeurycea mixteca</i>	Present	1		7	60	27

a gastropod. Whether or not their sister species is found in the same unusual habitat needs to be confirmed by finding gastropods infected with nematodes. Prevalence and abundance values, albeit low, prevent any expectation that the new species is actually a parasite of gastropods. Members of *Angiostoma* seem to be parasites primarily of gastropod mollusks (see Table 1) (see Morand et al., 2004), with a few species in amphibians that shares the same habitat with gastropods. The presence of a species of *Angiostoma* in a marine turtle (*C. caretta*) represents a very unusual finding, and future taxonomic papers will need to clarify the status of this species; however, our

observation of the type-specimens (USNPC No. 095696) of this species indicate they belong to *Angiostoma*.

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Literature cited

- Bursey, C. R. and S. R. Goldberg. 2000. *Angiostoma onychodactyla* sp. n. (Nematoda: Angiostomatidae) and other intestinal helminths of the Japanese clawed salamander, *Onychodactylus japonicus* (Caudata: Hynobiidae), from Japan. *Comparative Parasitology* 67:60-65.
- Bursey, C. R. and C. A. Manire. 2006. *Angiostoma carettae* n. sp. (Nematoda: Angiostomatidae) from the loggerhead sea turtle *Caretta caretta* (Testudines: Cheloniidae), Florida, U.S.A. *Comparative Parasitology* 73:253-256.
- Canseco-Márquez, L. and G. Gutiérrez-Mayén. 2005. New species of *Pseudoeurycea* (Caudata: Plethodontidae) from the mountains of the Mixteca Region of Oaxaca, Mexico. *Journal of Herpetology* 39:181-185.
- Chitwood, B.G. 1933. On some nematodes of the superfamily Rhabditoidea and their status as parasites of reptiles and amphibians. *Journal of the Washington Academy of Sciences* 23:508-520.
- Emery, M. B. and J. E. Joy. 2000. Endohelminths of the Ravine Salamander, *Plethodon richmondi*, from Southwestern West Virginia, USA. *Comparative Parasitology* 67:133-135.
- Falcón-Ordaz, J., J. C. Winfield-Pérez, B. Mendoza-Garfias, G. Parra-Olea and G. Pérez-Ponce de León. 2007. *Cosmocerca acanthurum* n. sp. (Nematoda: Cosmocercidae) in *Pseudoeurycea leprosa* and *Chiropterotriton orculus* from the Transmexican Volcanic Belt, central Mexico, with a checklist of the helminth parasites of plethodontid salamanders. *Zootaxa* 1434:27-49.
- Morand, S., M. J. Wilson and D. M. Glen. 2004. Nematodes (Nematoda) parasitic in terrestrial gastropods. In *Natural enemies of terrestrial molluscs*, G. M. Barker (ed.). CABI, Wallingford. p. 525-557.
- Parra-Olea, G. 2002. Phylogenetic relationships of the salamander of the genus *Pseudoeurycea* (Caudata: Plethodontidae). *Molecular Phylogenetics and Evolution* 22:234-246.
- Parra-Olea, G., L. Canseco-Márquez and M. García-París. 2004. A morphologically distinct new species of *Pseudoeurycea* (Caudata: Plethodontidae) from the Sierra Madre Oriental of Puebla, México. *Herpetologica* 64:78-84.
- Parra-Olea, G., M. García-París, M., J. Hanken and D. B. Wake. 2005. Two new species of *Pseudoeurycea* (Caudata: Plethodontidae) from the mountains of northern Oaxaca, México. *Copeia* 2005:461-469.
- Wiens, J. J., G. Parra-Olea, M. García-París and D. B. Wake. 2007. Phylogenetic history explains elevational biodiversity patterns in tropical salamanders. *Proceedings of the Royal Society of London B* 274:919-928.
- Windfield-Pérez J. C., G. Parra-Olea and L. Hernández-Zárate. 2007. Registro de *Pseudoeurycea mixteca* (Caudata: Plethodontidae) en una cueva de Tehuacán, Puebla. *Revista Mexicana de Biodiversidad* 78:493-495.