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MOLECULAR PHYLOGENY OF *HAEMATOLOECHUS* LOOSS, 1899 (DIGENEA: PLAGIORCHIIDAE), WITH EMPHASIS ON NORTH AMERICAN SPECIES

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ABSTRACT: Phylogenetic hypothesis of 23 populations corresponding to 18 species of the digenean *Haematoloechus* from America, Europe, and Africa, based on ribosomal DNA 28S partial sequences (~890 bp), is presented. Genetic divergence between the in-group and the out-groups ranged from 9.7 to 14.5% and within the in-group, from 0.9 to 12.2%. Eight most parsimonious trees 569 steps long were obtained, with a consistency index of 72%. Groups in the tree are not congruent with those in previous classification schemes of species in the genus, based on a small number of morphological characters. For this subset of *Haematoloechus* species, plesiomorphic hosts are species of *Rana*, with 2 colonizations to other amphibian groups. African species appear to have diverged after the separation of Gondwana and Laurasia. Therefore, South American species should appear as the closest relatives of African species when included in the analysis. The evidence presented suggests an ancestral wide distribution of North American representatives of the group, followed by successive contraction, amplification, and fragmentation of ranges and speciation events as a result of the intense volcanic activity in the central part of Mexico since the late Tertiary, the drying climate of western and central United States and northwestern Mexico from the early Eocene to the Pleistocene, and the glaciation during the Pleistocene.

Haematoloechus spp., the frog lung flukes, include some of the most common digeneans inhabiting frogs. More than 50 species have been described around the world, and their taxonomy has been highly controversial. Various authors have proposed different taxonomic schemes, grouping them in subgenera or proposing new genera to accommodate them (Odening, 1960; Skrjabin and Antipin, 1962; Yamaguti, 1971; Prudhoe and Bray, 1982). The cosmopolitan distribution of this genus and its host species (anurans and caudates) suggests a very ancient origin for the group. This was corroborated by a recent study of phylogenetic relationships among Holarctic species of *Haematoloechus* using ribosomal DNA (rDNA) sequences that evidenced the existence of clades with an origin predating the breakup of Laurasia (Snyder and Tkach, 2001). Great morphological divergence could be expected among species in the genus, considering their ancient origin and diversification; nevertheless, a similar general morphology and high intraspecific variation of some particular characters have led several authors to question the value of morphological traits in the recognition of *Haematoloechus* species or groups (Prokopic and Krivanek, 1974; Kennedy, 1980a, 1980b, 1981). All these features make the genus a highly desirable model system for assessing the utility of DNA sequences in solving taxonomic and phylogenetic problems (León-Règagnon et al., 1999, 2001; Snyder and Tkach, 2001; León-Règagnon and Paredes-Calderón, 2002).

In the present study, we update and expand the nucleotide sequence database for *Haematoloechus* species, using sequences of a variable region of the large subunit (LSU) of the rDNA to construct a phylogenetic hypothesis of species of *Haematoloechus*, evaluate morphological characters traditionally used to group species, and examine the biogeographic and coevolutionary patterns indicated by this subset of the genus.

MATERIALS AND METHODS

Collecting localities and hosts are detailed in Table I. Worms were identified in vivo; some samples were fixed in hot 4% formalin and

preserved in 70% ethanol for morphological study and comparison. Samples for molecular work were preserved in absolute ethanol. Snyder and Tkach (2001) expressed some concern about the quality of the sequences published by León-Règagnon et al. (1999). Attending this concern, we resequenced some of those isolates, now using automated sequencing instead of manual sequencing, the method used to generate the original sequences (Table I). Standard phenol extraction methods were used to recover DNA from entire worms (single specimens). Laboratory protocols followed Hillis et al. (1996) and Palumbi (1996). Polymerase chain reaction (PCR) was used for amplifying the 5' end of the 28S rRNA gene (~890 bp), including the D1 variable region. Amplification and sequencing were performed using the primers 28Sy 5'cta acc agg att ccc tca gta acg geg agt 3' (forward) and 28Sz 5'aga ctc ctt ggt ccg tgt ttc aag ac 3' (reverse) (Hillis and Dixon, 1991). The amplification program consisted of 1 min at 94 C followed by 35 cycles of 30 sec at 92 C, 30 sec at 50 C, and 1 min at 72 C, followed by 4 min at 72 C for final elongation. PCR products were sequenced directly on an ABI Prism 310 automated DNA sequencer. Sequences are available in GenBank with the accession numbers listed in Table I. Additional sequences for 10 *Haematoloechus* spp. isolates and for the 3 out-groups were obtained from previous publications (Tkach et al., 2000; Snyder and Tkach, 2001; León-Règagnon and Paredes-Calderón, 2002). Sequences were aligned visually using the computer program Bioedit (Hall, 1999). All analyses were conducted using PAUP 4.0b10 (Swofford, 2002). An uncorrected distance matrix was obtained for the pairs of examined sequences. Phylogenetic signal in the data set was estimated using the gI statistic (Hillis and Huelsenbeck, 1992) with 10,000 randomly generated trees. An unweighted maximum parsimony (MP) analysis using a branch and bound search was performed considering character states as unordered and gaps as missing data or as a fifth base. A nonparametric bootstrap (BTP) with 1,000 replicates was run to evaluate the robustness of the clades.

RESULTS

Sequences

The fragment of the ribosomal gene 28S for the 26 examined sequences varied from 852 to 884 bp, resulting in an alignment of 889 positions. Aligned sequences are available at TreeBASE with the accession number M1435. Genetic divergence between the in-group and the out-groups ranged from 9.7 to 14.5% and within the in-group, from 0.9 to 12.2%. In some cases, the rDNA sequences from species of the in-group are less similar to each other (*H. micrurus* vs. *H. breviplexus*, 12.2%) than those from species from the in-group and the out-group (*H. exoterorchis* vs. *Haplometra cylindracea*, 9.7%).

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TABLE I. Specific designations, hosts, and localities of specimens used in this study and GenBank accession numbers or references for corresponding sequences.

Species	Host species	Locality	GenBank no.	Vouchers
<i>Haematoloechus abbreviatus</i>	<i>Bombina variegata</i>	Zakarpatska Region, Ukraine	AF184251*	
<i>H. asper</i>	<i>Rana arvalis</i>	Ivano-Frankivsk Region, Ukraine	AF151934*	
<i>H. breviplexus</i> (M)	<i>R. luteiventris</i>	Glacier National Park, Montana	AF531856	CNHE4660
<i>H. breviplexus</i> (A)	<i>R. catesbeiana</i>	Cochise County, Arizona	AF387800†	
<i>H. coloradensis</i>	<i>R. dunnii</i>	Lake Pátzcuaro, Michoacán, México	AF531867§	CNHE4661
<i>H. cf. complexus</i> (L)	<i>R. montezumae</i>	Lerma Wetlands, Estado de México	AF532138§	CNHE3374-78
<i>H. cf. complexus</i> (T)	<i>R. vaillanti</i>	Los Tuxtlas, Veracruz, México	AF531857	CNHE4086-87
<i>H. complexus</i>	<i>R. blairi</i>	Gage County, Nebraska	AF387797†	
<i>H. danbrooksi</i>	<i>R. vaillanti</i>	Los Tuxtlas, Veracruz, México	AF479652‡	CNHE4112,4151 USNPC92220
<i>H. exoterorichis</i>	<i>R. occipitalis</i>	Sierra Leone	AF531858	CNHE4662
<i>H. floedae</i>	<i>R. catesbeiana</i>	Fort Stewart, Georgia	AF531859	CNHE4663
<i>H. illimis</i>	<i>R. montezumae</i>	Lerma Wetlands, Estado de México	AF531860§	CNHE3381-83
<i>H. longiplexus</i> (N1)	<i>R. catesbeiana</i>	Neligh, Nebraska	AF531861§	CNHE4664
<i>H. longiplexus</i> (N2)	<i>R. catesbeiana</i>	Gage County, Nebraska	AF387801†	
<i>H. macrorrichis</i>	<i>R. montezumae</i>	Lerma Wetlands, Estado de México	AF531862§	CNHE3394
<i>H. mediotplexus</i> (N)	<i>R. pipiens</i>	Neligh, Nebraska, U.S.A.	AF531863§	CNHE4665
<i>H. mediotplexus</i> (W)	<i>R. pipiens</i>	Winnebago County, Wisconsin	AF387799†	
<i>H. meridionalis</i>	<i>R. vaillanti</i>	Rio Pizote, Guanacaste, Costa Rica	AF531864	CNHE4199-4203 USNPC91036-37
<i>H. micrurus</i>	<i>R. occipitalis</i>	Sierra Leone	AF531865	CNHE4666
<i>H. parviplexus</i>	<i>R. catesbeiana</i>	40 km south of Humboldt, Nebraska	AF479653‡	CNHE4405
<i>H. pulcher</i>	<i>R. montezumae</i>	Lerma Wetlands, Estado de México	AF531866§	
<i>H. variegatus</i>	<i>R. ridibunda</i>	Ivano-Frankivsk Region, Ukraine	AF151916*	CNHE3398-3400
<i>H. variotplexus</i>	<i>R. clamitans</i>	Winnebago County, Wisconsin	AF387798†	
<i>Brachycoelium salamandrae</i>	<i>Salamandra salamandra</i>	Zakarpatska Region, Ukraine	AF151935*	
<i>Haplometra cylindracea</i>	<i>R. arvalis</i>	Zakarpatska Region, Ukraine	AF151933*	
<i>Plagiorchis vespertilionis</i>	<i>Myotis daubentoni</i>	Sumy Region, Ukraine	AF151931*	

* Tkach et al. (2000).

† Snyder and Tkach (2001).

‡ León-Régagnon and Paredes-Calderón (2002).

§ Sequences obtained from the same isolates referred in León-Régagnon et al. (1999).

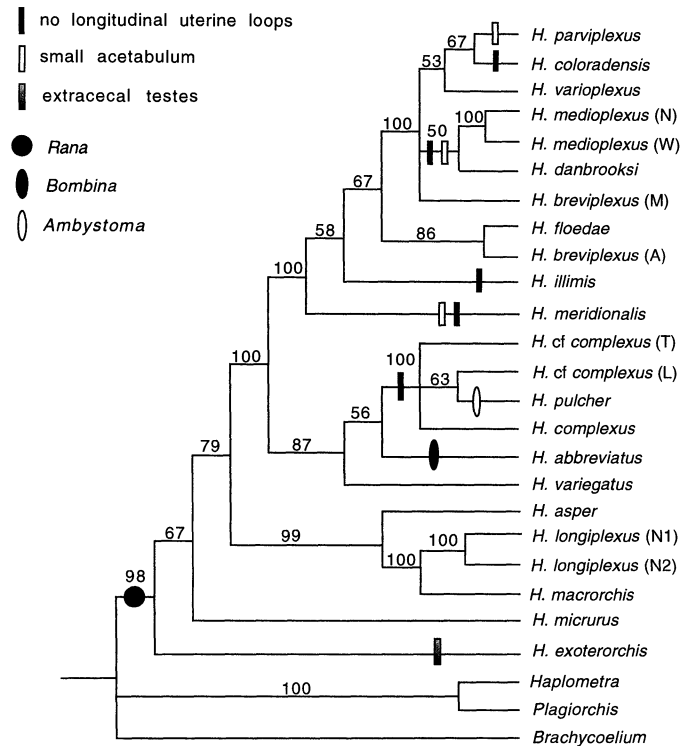


FIGURE 1. Strict consensus of the 8 most parsimonious trees for the fragment of the rDNA 28S gene examined in this study, showing morphological traits and preferential hosts mapped on the tree. Names correspond to those assigned in Table I. Numbers above branches indicate BTP support.

Phylogenetic analysis

The data set presented a significant phylogenetic signal ($gI = -0.646$, $P > 0.01$; mean \pm SD tree length $1,171.44 \pm 42.06$, range 958–1,260). The MP analysis performed with the 26 taxa provided 262 (308 considering gaps as a fifth base) variable characters, 173 (181) of which were parsimoniously informative. Four (8) equally parsimonious trees were obtained, 486 (569) steps long, with a consistency index of 0.69 (0.72) and a retention index of 0.82.

The strict consensus of the 4 most parsimonious trees obtained considering gaps as missing data (Fig. 1) shows *Haematoloechus exoterorchis* as the sister species to the rest of the in-group. In the next node, *H. micrurus* appears as the basal species for 2 major clades. One of them (BTP = 99) includes *H. asper* as the sister species of *H. longiplexus* and *H. macrorchis*. The second clade (BTP = 100) comprises 2 groups; 1 of them includes 2 European species (*H. abbreviatus* and *H. variegatus*) basal to a polytomy comprising specimens assigned to the North American *H. complexus* group. The second group includes only American species; the Central American species *H. meridionalis* is basal, followed by 1 Mexican species (*H. illimis*) with a low BTP support (58). The next clade is also weakly supported, showing *H. floedae* + *H. breviplexus* (of Snyder and Tkach, 2001) as the sister taxa of a polytomy formed by North American species. Within this clade, *H. danbrooksi* appears as sister species of *H. medioplexus*, and *H. varioplexus* is the sister species of *H. parviplexus* + *H. coloradensis*. Poor resolution and low BTP support were obtained in

this part of the tree. The consensus tree of the 8 most parsimonious trees obtained considering gaps as a fifth base is identical, except for the North American species clade, which is less resolved.

DISCUSSION

Sequences divergence

Snyder and Tkach (2001) suggested that the amount of variation in sequences of rDNA of several species of *Haematoloechus* from Mexico and the United States observed by León-Règagnon et al. (1999) was the result of errors in the methodology used by those authors, and they recommended caution in the use of those data by others. Following their suggestion, we compared LSU sequences from León-Règagnon et al. (1999) with those obtained in this study. León-Règagnon et al. (1999) used manual sequencing in their study; the differences found with the sequences we obtained in this study from the same samples, but using automated sequencing, apparently are the result of misinterpretation of autoradiographs. Snyder and Tkach (2001) expressed their concern about the great variation shown between 2 sequences of *H. longiplexus* from different localities in the study of León-Règagnon et al. (1999), compared with the small variation found by these authors in different populations of 2 European species. León-Règagnon et al. (1999) considered 2 populations from Nebraska and Central Mexico to belong to *H. longiplexus*. Further research on sequences of specimens from distant populations of *H. floedae* have shown them to be identical or to differ only in 2 sites. These observations, together with the evidence presented by Snyder and Tkach (2001) about the low divergence among populations of *H. longiplexus*, suggest that the synonymy of *H. macrorchis* with *H. longiplexus* suggested by León-Règagnon et al. (1999) was premature and that they are actually valid species that are morphologically very similar.

In the LSU fragment that we studied herein, we found no genetic variation between 2 close populations of *H. longiplexus* (both in the state of Nebraska) and little genetic divergence between 2 populations of *H. medioplexus* from Nebraska and Wisconsin (0.3%). The sequence assigned to *H. breviplexus* from Arizona by Snyder and Tkach (2001) differs by 0.2% from our sequence of *H. floedae* from Georgia and by 3.5% from the sequence of *H. breviplexus* from Montana.

Haematoloechus breviplexus, originally described from Ontario, Canada, is morphologically similar to *H. floedae*, originally described from Texas. Various authors have considered *H. floedae* as a junior synonym of *H. breviplexus* (Odening, 1960; Kennedy, 1981). We examined the voucher specimen identified as *H. breviplexus* by Snyder and Tkach (2001) (USNPC 91507) and corroborated that it actually belongs to *H. floedae*. It differs from *H. breviplexus* in the shape of the testes, the more anterior uterine longitudinal loops in *H. floedae*, and the distribution of the vitelline follicles, which are smaller and more numerous in *H. floedae*, reaching different levels on each side of the body. According to the morphological and molecular data presented herein, *H. floedae* is a valid species, and the sequence presented by Snyder and Tkach (2001) for *H. breviplexus* probably came from a misidentified specimen of *H. floedae*. Although the known geographic distribution of *H. floedae* is in the south-

eastern United States, wide dispersion of the definitive host due to human activities could have widened its range.

In our study, the amount of genetic divergence is more similar among sequences of different populations previously assigned to *H. complexus* than among sequences from specimens assigned to different species (i.e., *H. coloradensis* and *H. parviplexus*), suggesting that Mexican isolates of this species could represent independent lineages. Nevertheless, with the information available to date, species limits are difficult to establish in this morphologically conservative group. Genetic variation among populations of the entire geographical range of the complex of species needs to be carefully explored.

The amount of divergence between some pairs of species of the in-group is strikingly high (*H. breviflexus* vs. *H. micrurus*, 12.2%), surpassing that observed between some species of the in-group and the out-group (*H. exoterorchis* and *Haplometra cylindracea*, 9.7%). In a phylogenetic study of the Plagiorchiata (Tkach et al., 2001), *Haplometra cylindracea* and European species of *Haematoloechus* appear in distantly related clades within the group. Nevertheless, those clades appear with a very low BTP support in Tkach et al.'s (2001) study, and it is possible that *Haematoloechus* and *Haplometra* are more closely related than they appear if additional evidence is evaluated. In any case, *H. exoterorchis* has been considered to be an independent genus, *Metahaematoloechus*, based on the position of the testes (Yamaguti, 1970), and the present study gives support to that proposal.

Morphological traits

Analysis of the phylogenetic history of *Haematoloechus* spp. is hindered by the absence of any phylogenetic analyses based on a large suite of morphological traits. Currently, a small number of morphological characters are used to classify *Haematoloechus* spp., and they do not support the groups in our phylogenetic hypothesis (Fig. 1). The arrangement of the uterine loops has been 1 of the most important taxonomic characters for differentiation of species and classification of the group. Several authors have considered that species without extracecal longitudinal uterine loops constitute a different genus, *Ostiolum* (Pratt, 1930; Odening, 1960; Skrjabin and Antipin, 1962; Yamaguti, 1971; Prudhoe and Bray, 1982). According to our analysis, *Haematoloechus* spp. is paraphyletic if species without extracecal longitudinal uterine loops are grouped in a different genus (Fig. 1). This assertion is in agreement with previous studies in which species without longitudinal uterine loops do not appear as closest relatives (León-Régagnon et al., 1999; Snyder and Tkach, 2001). Size of the acetabulum relative to oral sucker has also been an important taxonomical feature in the genus. In our analysis, species with acetabula less than 50% the size of the oral sucker are all included in the clade of North American species, although they do not appear as a monophyletic group within this clade (Fig. 1). BTP support of this part of the tree is low. Only by the inclusion of more taxa, including those without acetabula (proposed to form an independent genus, *Neohaematoloechus* Odening, 1960), and the use of additional molecular markers will we be able to test the validity of this character as indicative of evolutionary lineages. *Haematoloechus exoterorchis* was suggested to constitute a different genus, *Metahaematoloechus*, characterized by having extracecal

testes (Yamaguti, 1971; Prudhoe and Bray, 1982). Our hypothesis is consistent with that classification scheme (although with a low BTP support). Representatives of *Skrjabinoeces* (European species with vitelline follicles limited to the anterior part of the body) need to be included in the analysis to test the value of the distribution of vitelline follicles for the classification of the group. Arrangement of the uterine loops and size of the acetabulum are apparently useful to distinguish species of the genus but do not represent the phylogenetic affinities within the group.

Host and geographical affinities

Mapping preferential hosts on the tree (Fig. 1) indicates that plesiomorphic hosts are species of *Rana* and colonization of members of *Bombina* (*H. abbreviatus*) or ambystomatids (if *H. pulcher* is a valid species) may have led to speciation events through host switching. Within *Rana*, no obvious coevolutionary pattern can be distinguished because several different lineages of frogs (Hillis and Davies, 1986) are infected by species of the same group of worms. The *H. complexus* group apparently has been limited to leopard frogs, but, again, it is necessary to perform further studies using more informative DNA regions for this taxonomic level to determine whether coevolutionary phenomena have occurred between these groups. *Haematoloechus* species are morphologically conservative, and it is possible that only with the use of molecular markers will we be able to understand whether we are dealing with 1, or several, different species parasitizing leopard frogs in Mexico.

Our hypothesis is also consistent with previous analysis suggesting an origin of the group predating the breakup of Pangea (Snyder and Tkach, 2001). North American, European, and African representatives of *Haematoloechus* do not form monophyletic groups (Fig. 2). According to our study, African species diverged early in the diversification of the group, after the breakup of Gondwana and Laurasia, during the late Triassic. To obtain a fuller picture of the evolution of this group, we need to include South American, Asian, and Australian species in the analysis. For example, Cordero and Vogelsang (1939) suggested a Nearctic origin for the South American species described during that period, assigning a North American ancestral species to each one of them. Their scenario implies many colonization events of North American species and subsequent speciation events in South America. If the hypothesis based on the small subset of species used in this and previous molecular studies is correct, we predict that South American species of *Haematoloechus* would appear as the closest relatives of African species when included in the analysis.

According to our observations on the Mexican and Central American species of *Haematoloechus*, the southernmost areas in the distribution of the majority of the Nearctic species that extend their distribution to Mexico (*H. coloradensis*, *H. complexus*) appear to be the Mesa Central, the southern part of Veracruz State, along the Atlantic coast, and the lowlands of Guerrero on the Pacific coast of Mexico (León-Régagnon et al., 1999; Pérez-Ponce de León et al., 2000; this study). In the Mesa Central and in the lowlands of both coasts, there appears to be a mixture of Nearctic species and endemics of southern Mexico and Central America (*H. coloradensis*, *H. complexus*, *H. floedae*, *H. macrorchis*, *H. illimis*, *H. danbrooksi*, *H. pulcher*, *H.*

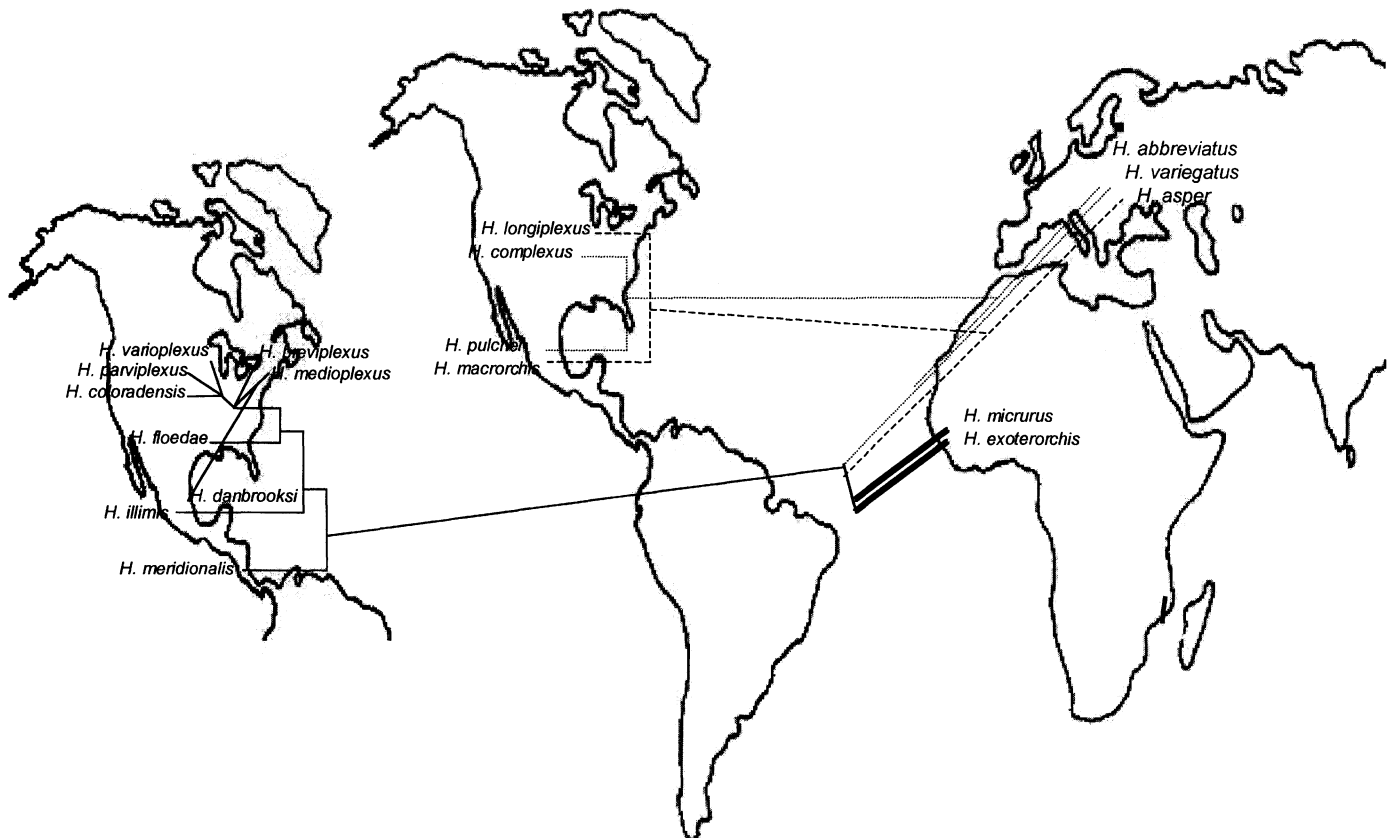


FIGURE 2. Strict consensus of the 8 most parsimonious trees superimposed on a map showing geographical distribution of species of *Haematoloechus* included in this study.

meridionalis). In our phylogenetic hypothesis, species from Middle America appear scattered in the tree, together with typically Nearctic species, in 1 case being basal to North American species (*H. meridionalis* and *H. illimis*) (Fig. 2). This evidence suggests a wide ancestral distribution of North American representatives of the group, followed by successive contraction, amplification, and fragmentation of ranges and speciation events. These activities were the result of intense volcanic activity in the central part of Mexico since the late Tertiary, the drying climate of western and central United States and northwestern Mexico from the early Eocene to the Pleistocene, and the glaciating event during the Pleistocene (Rosen, 1978; Fergusuía-Villafranca, 1998). Regions that maintained a stable climate during these events include the southeastern United States, the Gulf of Mexico coast, the Yucatan Peninsula, and parts of extreme western North America. It has been recognized that several groups of organisms show a geographical distribution more or less congruent with these areas as a result of the retraction of their original range during those periods of geologic and climatic change (Rosen, 1978; Byerly, 1991). In our analysis of *Haematoloechus* spp., we recognize some taxa that follow this geographical pattern, i.e., *H. floedae*, which is distributed in the southeastern United States and is found in the Yucatan Peninsula. *Haematoloechus longiplexus* is distributed in the eastern United States; its sister species, *H. macrorchis*, is found in eastern Central Mexico. *Haematoloechus coloradensis* is found from the western United States to Central Mexico and the Pacific coast. Additional inventory in Central America may

produce more species of *Haematoloechus*, helping us to complete this part of the story. In addition, we believe that the use of sequence data from mitochondrial genes may give us information at a lower taxonomic level, i.e., the actual species and geographic ranges of members of the *H. complexus* group.

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LITERATURE CITED

- BYERLY, G. R. 1991. Igneous activity. In *The Gulf of Mexico basin: The geology of North America*, vol. J., A. Salvador (ed.). Geological Society of America, Boulder, Colorado, p. 91–108.
- CORDERO, E. H., AND E. G. VOGELSANG. 1939. Nuevos Tremátodos, I. Dos Especies del Género *Pneumonoeces* Looss, del pulmón de *Rana palmipes* Spix, de Venezuela. *Revista de Medicina Veterinaria y Parasitología* 1: 173–179.

- FERRUSQUÍA-VILAFRANCA, I. 1998. Geología de México: Una sinopsis. In *Diversidad biológica de México: Orígenes y distribución*, T. P. Ramamoorthy, R. Bye, A. Lot, and J. Fa (eds.). Universidad Nacional Autónoma de México, Mexico, Mexico, p. 3–108.
- HALL, T. A. 1999. Bioedit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41**: 95–98.
- HILLIS, D. M., AND S. K. DAVIES. 1986. Evolution of ribosomal DNA: Fifty million years of recorded history in the frog genus *Rana*. *Evolution* **40**: 1275–1288.
- , AND T. M. DIXON. 1991. Ribosomal DNA: Molecular evolution and phylogenetic inference. *The Quarterly Review of Biology* **66**: 411–453.
- , AND J. P. HUELSENBECK. 1992. Signal, noise and reliability in molecular phylogenetic analyses. *Journal of Heredity* **83**: 189–195.
- , B. K. MABLE, AND C. MORITZ. 1996. Nucleic acids IV: Sequencing and cloning. In *Molecular systematics*, D. M. Hillis, C. Moritz, and B. K. Mable (eds.). Sinauer Associates, Sunderland, Massachusetts, p. 321–383.
- KENNEDY, M. J. 1980a. Host-induced variations in *Haematoloechus butensis* (Trematoda: Haematoloechidae). *Canadian Journal of Zoology* **58**: 427–442.
- . 1980b. Geographical variation in some representatives of *Haematoloechus*, Looss, 1899 (Trematoda: Haematoloechidae) from Canada and the United States. *Canadian Journal of Zoology* **58**: 1151–1167.
- . 1981. A revision of species of the genus *Haematoloechus* Looss, 1899 (Trematoda: Haematoloechidae) from Canada and the United States. *Canadian Journal of Zoology* **59**: 1836–1846.
- LEÓN-RÉGAGNON, V., D. R. BROOKS, AND G. PÉREZ-PONCE DE LEÓN. 1999. Differentiation of Mexican species of *Haematoloechus* Loss, 1899 (Digenea: Plagiorchiformes): Molecular and morphological evidence. *Journal of Parasitology* **85**: 935–946.
- , ———, AND D. A. ZELMER. 2001. Morphological and molecular description of *Haematoloechus meridionalis* n. sp. (Digenea: Plagiorchioidea: Haematoloechidae) from *Rana vaillanti* Brocchi of Guanacaste, Costa Rica. *Journal of Parasitology* **87**: 1423–1427.
- , AND E. L. PAREDES-CALDERÓN. 2002. *Haematoloechus danbrooksi* n. sp. (Digenea: Plagiorchioidea), from *Rana vaillanti* of Los Tuxtlas, Veracruz, Mexico. *Journal of Parasitology* **88**: 1215–1221.
- ODENING, K. 1960. Plagiorchiidae III. (Haematoloechinae) und Omphalometrinae. In *Das Tierreich. Eine Zusammenstellung und Kennzeichnung der rezenten Tierformen*, R. Mertens and W. Hennig (eds.). Walter de Gruyter & Co., Berlin, Germany, p. 1–75.
- PALUMBI, S. R. 1996. Nucleic acids II: The polymerase chain reaction. In *Molecular systematics*, D. M. Hillis, C. Moritz, and B. K. Mable (eds.). Sinauer Associates, Sunderland, Massachusetts, p. 205–247.
- PÉREZ-PONCE DE LEÓN, G., V. LEÓN-RÉGAGNON, L. GARCÍA-PRIETO, U. RAZO-MENDIVIL, AND A. SÁNCHEZ-ALVAREZ. 2000. Digenean fauna of amphibians from Central Mexico: Nearctic and Neotropical influences. *Comparative Parasitology* **67**: 92–104.
- PRATT, H. S. 1930. Description of four distomes. In *Mark Anniversary Volume*, G. H. Parker (ed.). Henry Holt Publishers, New York, New York, p. 25–38.
- PROKOPIC, J., AND K. KRIVANEK. 1974. Trematodes of the genus *Haematoloechus* Looss, and their variability. *Helminthologia* **15**: 779–802.
- PRUDHOE, S., AND R. A. BRAY. 1982. Platyhelminth parasites of the Amphibia. Oxford University Press, Oxford, U.K., 217 p.
- ROSEN, D. E. 1978. Vicariant patterns and historical explanation in biogeography. *Systematic Zoology* **27**: 159–188.
- SKRJABIN, K. I., AND D. N. ANTIPIN. 1962. The superfamily Plagiorchioidea Dollfus, 1930. In *Trematodes of animals and man*, vol. 20, K. I. Skrjabin (ed.). Akademy Nauk, CCCP, Moscow, Russia, p. 47–163. [In Russian.]
- SNYDER, S., AND V. TKACH. 2001. Phylogenetic and biogeographical relationships among some holarctic frog lung flukes (Digenea: Haematoloechidae). *Journal of Parasitology* **87**: 1433–1440.
- SWOFFORD, D. L. 2002. PAUP*: Phylogenetic analysis using parsimony (*and other methods), version 4. Sinauer Associates, Sunderland, Massachusetts.
- TKACH, V., J. PAWLOWSKI, AND J. MARIAUX. 2000. Phylogenetic analysis of the suborder Plagiorchiata (Platyhelminthes, Digenea) based on partial 18S rDNA sequences. *International Journal for Parasitology* **30**: 83–93.
- YAMAGUTI, S. 1971. Synopsis of digenetic trematodes of vertebrates. Keigaku Publishing Co., Tokyo, Japan, 1074 p.