University of Nebraska - Lincoln

DigitalCommons@University of Nebraska - Lincoln

Faculty Publications from the Harold W. Manter
Laboratory of Parasitology

Parasitology, Harold W. Manter Laboratory of

2011

ERECTION OF *IBIRHYNCHUS* GEN. NOV. (ACANTHOCEPHALA: POLYMORPHIDAE), BASED ON MOLECULAR AND MORPHOLOGICAL DATA

Martin Garcia-Varela

Instituto de Investigaciones Biomedicas Mexico, garciav@servidor.unam.mx

Gerardo Pérez-Ponce de León

Universidad Nacional Autónoma de México

Francisco J. Aznar

Universitat de Valencia

Steven A. Nadler

University of California - Davis, sanadler@ucdavis.edu

Follow this and additional works at: <https://digitalcommons.unl.edu/parasitologyfacpubs>

Part of the [Parasitology Commons](#)

Garcia-Varela, Martin; Pérez-Ponce de León, Gerardo; Aznar, Francisco J.; and Nadler, Steven A., "ERECTION OF *IBIRHYNCHUS* GEN. NOV. (ACANTHOCEPHALA: POLYMORPHIDAE), BASED ON MOLECULAR AND MORPHOLOGICAL DATA" (2011). *Faculty Publications from the Harold W. Manter Laboratory of Parasitology*. 719.

<https://digitalcommons.unl.edu/parasitologyfacpubs/719>

This Article is brought to you for free and open access by the Parasitology, Harold W. Manter Laboratory of at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Faculty Publications from the Harold W. Manter Laboratory of Parasitology by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

ERECTION OF *IBIRHYNCHUS* GEN. NOV. (ACANTHOCEPHALA: POLYMORPHIDAE), BASED ON MOLECULAR AND MORPHOLOGICAL DATA

Martín García-Varela, Gerardo Pérez-Ponce de León, Francisco J. Aznar*, and Steven A. Nadler†

Departamento de Zoología, Instituto de Biología, Universidad Nacional Autónoma de México, Avenida Universidad 3000; Ciudad Universitaria, C.P. 04510, Distrito Federal, México. e-mail: garciav@servidor.unam.mx

ABSTRACT: The genus *Southwellina* is composed of 3 described species, i.e., *S. hispida* (the type species), *S. dimorpha*, and *S. macracanthus*. All 3 are endoparasites of fish-eating birds that have worldwide distributions. Morphologically, the genus is characterized by possessing a short and compact trunk, 2 fields of spines in the anterior region of the trunk (in at least 1 sex), a short cylindrical proboscis (sometimes with a swollen region armed with numerous longitudinal rows of hooks), a double-walled proboscis receptacle, and 4 tubular cement glands in males. In the current study, specimens identified as *S. dimorpha* were collected from *Eudocimus albus* (white ibis), the type host from the Gulf of Mexico. Sequences of 2 nuclear genes (small subunit [SSU] and long subunit [LSU] ribosomal DNA) and 1 mitochondrial gene (cytochrome *c* oxidase subunit 1 [cox 1]) of *S. dimorpha* and *S. hispida* were obtained and used to reconstruct the phylogenetic relationships of both species with respect to published sequences of 11 species representing 6 genera of Polymorphidae. Maximum parsimony (MP) and maximum likelihood (ML) analyses of the concatenated data set (SSU + LSU + cox 1) were identical in depicting *Southwellina* as paraphyletic, indicating that the genus should be revised. The MP and ML trees identified *S. hispida* as a sister to *Polymorphus brevis*, whereas *S. dimorpha* was a sister to *Hexaglandula corynosoma*. Morphologically, *S. dimorpha* is distinct from *H. corynosoma*, which is characterized by a short trunk with 1 field of spines in the anterior part of the trunk in both genders, and males with 6 tubular cement glands. The genetic divergence estimated from a concatenated data set between 2 isolates of *S. hispida* and *S. dimorpha* ranged from 10.7 to 11.0%. This range of genetic divergence is similar to that found among other genera of Polymorphidae, which extends from 6.0 to 12.0%. *Southwellina dimorpha* differs from *S. hispida* in the shape of the proboscis and the presence of 1 field of spines (*S. dimorpha*) versus 2 fields (*S. hispida*) on the anterior region of the trunk in females. Based on the phylogenetic position of *S. dimorpha* within Polymorphidae, coupled with levels of genetic divergence and, more importantly, the morphological and ecological (host specificity) differences, we propose the erection of a new genus to accommodate *S. dimorpha*.

Members of Polymorphidae Meyer, 1931 are intestinal parasites of marine mammals, fish-eating birds, and waterfowl, with a worldwide distribution. Their life cycles typically include a crustacean (amphipod, copepod, or decapod) as an intermediate host and may include fish, snakes, frogs, or toads as paratenic hosts (Schmidt, 1985; Hoberg, 1986; Nickol et al., 1999, 2002). Polymorphids include approximately 127 species, classified into 11 genera (Schmidt, 1973; Dimitrova and Goergiev, 1994; Nickol et al., 1999, 2002; Aznar et al., 2006). Currently, *Southwellina* Witenberg, 1932 is a genus composed of 3 described species (Schmidt, 1973; Amin, 1985). The type species, *S. hispida* Van Cleave 1925, is a parasite of fish-eating birds, with a worldwide distribution (Schmidt, 1973; Courtney and Forrester, 1974; Richardson and Cole, 1997; Dezfuli et al., 2002; Norman et al., 2003; Font, 2007; García-Varela et al., 2009). *Southwellina macracanthus* Ward and Winter, 1952 was described from cystacanths of marine fishes obtained from the Pacific Coast of the United States. *Southwellina dimorpha* Schmidt, 1973 was described from the intestine of *Eudocimus albus* (white ibis) from Florida, and recently it was found in the intestine of *Grus americana* (whooping crane) (Richardson and Font, 2006). Species of *Southwellina* are characterized by possessing a short and compact trunk, 2 fields of spines in the anterior region of the trunk (in at least 1 sex), a short cylindrical proboscis armed with numerous longitudinal rows of hooks, a double-walled and cylindrically shaped proboscis receptacle, and lemnisci that are longer than the proboscis receptacle. Males possess 2 testes in the

anterior part of the trunk and 4 tubular cement glands (Schmidt, 1973).

In the present study, specimens identified as *S. dimorpha* and *S. hispida* were used for molecular phylogenetic inference in an analysis including other members of Polymorphidae. Sequences of the small (SSU) and large (LSU) subunit nuclear ribosomal DNA and a region of the mitochondrial cytochrome *c* oxidase subunit I (cox 1) gene were obtained for both species of *Southwellina*. Sequences of both species were aligned with previously acquired data (see García-Varela et al., 2009) that included representatives of 6 genera of Polymorphidae (*Polymorphus* Lühe, 1911, i.e., *Andracantha* Schmidt, 1975, *Pseudocorynosoma* Aznar, Pérez-Ponce de León and Raga, 2006, *Corynosoma* Lühe, 1904, *Hexaglandula* Petrochenko, 1950, *Profilicollis* Meyer, 1931), and 3 outgroup taxa, with the aim of examining the phylogenetic position and the sister-group relationships of *S. hispida* and *S. dimorpha* within Polymorphidae. In addition, a detailed morphological comparison was made between specimens of *S. hispida* and *S. dimorpha* to confirm the molecular findings.

MATERIALS AND METHODS

Specimens and DNA isolation

Adult acanthocephalans were collected from 5 white ibis (*Eudocimus albus*) from 2 localities in the Gulf of Mexico, i.e., Los Chivos, Veracruz (18°56'13"N, 95°58'08"W) and Catemaco, Veracruz (18°25'N, 95°07'W). Worms were washed 3 times in 0.9% (w/v) saline, preserved in absolute ethanol, and stored at 4 °C. For morphological identification, some specimens were stained with Meyer's paracarmine, cleared with methyl salicylate, and mounted on slides using Canada balsam. Parasites collected from *Eudocimus albus* were identified as *S. dimorpha* by conventional morphological criteria following keys of Yamaguti (1963) and Petrochenko (1958) and the original and revised descriptions of the species (Schmidt, 1973). Avian definitive hosts were identified using the field guides of Howell and Webb (1995) and the American Ornithologists' Union (1998). Voucher specimens were deposited at the Colección Nacional de Helmintos, Instituto de Biología, UNAM, México City, México (Table I).

Received 25 September 2009; revised 8 April 2010, 20 September 2010; accepted 23 September 2010.

* Departamento de Biología Animal, Instituto Cavanilles de Biodiversidad y Biología Evolutiva, Universitat de Valencia, Burjassot, Valencia, Spain.

† Department of Nematology, University of California, Davis, California 95616-8668.

DOI: 10.1645/GE-2350.1

TABLE I. Specimen information and GenBank accession numbers for specimens studied in this work. Sequences marked with an asterisk (*) were obtained in this study. ND, not determined. The numbers 1 and 2 correspond with the same numbers in Figures 1 and 2.

Species	Host	Locality	Vouchers (CNHE)	GenBank accession Cox	GenBank accession SSU	GenBank accession LSU
<i>Andracantha gravida</i>	<i>Phalacrocorax auritus</i>	Yucatán, México	5997	EU267822	EU267802	EU267814
<i>Corynosoma enhydri</i>	<i>Enhydra lutris</i>	Monterey Bay, California, USA	3429	DQ089719	AF001837	AY829107
<i>Corynosoma magdaleni</i>	<i>Phoca hispida saimensis</i>	Lake Saimaa, Finland		EF467872	EU267803	EU267815
<i>Corynosoma strumosum</i>	<i>Phoca vitulina</i>	Monterey Bay, California, USA		EF467870	EU267804	EU267816
<i>Ibhirhynchus dimorpha</i>	<i>Eudocimus albus</i>	Veracruz, México	6164	GQ981438*	GQ981436*	GQ981437*
<i>Hexaglandula corynosoma</i>	<i>Nyctanassa violacea</i>	Veracruz, México	5765	EF467869	EU267808	EU267817
<i>Polymorphus brevis</i>	<i>Nycticorax nycticorax</i>	Michoacán, México	5778	DQ089717	AF064812	AY829105
<i>Polymorphus minutus</i>	<i>Gammarus pulex</i>	Dijon, France		EF467865	EU267806	EU267819
<i>Profilicollis altmani</i>	<i>Enhydra lutris</i>	Monterey Bay, California, USA	5777	DQ089720	AF001838	AY829108
<i>Profilicollis botulus</i> ¹	<i>Somateria mollissima</i>	Denmark	5768	EF467862	EU267805	EU267818
<i>Profilicollis botulus</i> ²	<i>Anas platyrhynchos</i>	USA		DQ089721	AF064815	AY829109
<i>Pseudocorynosoma constrictum</i>	<i>Anas clypeata</i>	Estado de México, México	5720	EU267820	EU267800	EU267812
<i>Pseudocorynosoma anatarium</i>	<i>Bucephala albeola</i>	Durango, México	5721	EU267821	EU267801	EU267813
<i>Southwellina hispida</i> ¹	ND	Baltic Sea, Finland		EF467866	EU267809	EU267810
<i>Southwellina hispida</i> ²	<i>Tigrisoma mexicanum</i>	Veracruz, México	5769	EF467867	EU267807	EU267811
<i>Centrorhynchus</i> sp.	<i>Falco peregrinus</i>	California, USA		DQ089716	AY830155	AY829104
<i>Gorgorhynchoides bullocki</i>	<i>Eugerres plumieri</i>	Quintana Roo, México		DQ089715	AY830154	AY829103
<i>Plagiorhynchus cylindraceus</i>	<i>Porcilio saber</i>	Dijon, France		DQ089724	AF001839	AY829102

Six adult acanthocephalans identified as *S. dimorpha* and *S. hispida* were prepared for scanning electron microscopy (SEM) using standard methods (Guillén-Hernández et al., 2008). SEM facilitated observations on anterior trunk spines and hooks of the proboscis. Illustrations of *S. dimorpha* and *S. hispida* were prepared with the aid of a drawing tube attached to the microscope. Measurements are given in millimeters (mm). For comparative purposes, 38 mature specimens (31 females and 7 males) of *S. hispida* collected from different bird species in the Gulf of Mexico and Pacific Ocean slopes were studied. In addition, vouchers of different species of polymorphids from the National Collection of Helminths (CNHE) (see García-Prieto et al., 2010), and the Harold W. Manter Laboratory of Parasitology, Lincoln, Nebraska (HWML), were studied as follows: *S. hispida* (CNHE 6716, 6720, 6735–6736, 6689–6691, HWML 34897, 34898, 34902, 34903), *S. dimorpha* (HWML 34731, 34732), *S. macracanthus* (HWML 34527, 34528), and *Hexaglandula corynosoma* Travassos, 1915 (CNHE 5765, 5771–5773).

Amplification and sequencing of DNA

Two specimens of *S. dimorpha* were used for DNA extraction; 1 individual was from Los Chivos, Veracruz, México, and the other from Catemaco, Veracruz, México. These individuals were digested overnight at 56 °C in a solution containing 10 mM Tris-HCl, pH 7.6, 20 mM NaCl, 100 mM Na₂EDTA, pH 8.0, 1% Sarkosyl, and 0.1 mg/ml proteinase K. After digestion, DNA was extracted from the supernatant using the DNAzol reagent (Molecular Research Center, Cincinnati, Ohio) according to the manufacturer's instructions.

Two regions of nuclear ribosomal DNA (rDNA) were amplified using the polymerase chain reaction (PCR). The near-complete SSU rDNA (~1,800 bp) was amplified in 1 fragment using the forward primer 5'-AGATTAAGCCATGCATGCGT and reverse primer 5'-GCAGGTT-CACCTACGAAA. The near-complete LSU rDNA (~2,900 bp) was amplified using 2 overlapping PCR fragments of 1,400–1,500 bp. Primers for LSU amplicon 1 were forward 5'-CAAGTACCGTGAGG-GAAAGTTGC and reverse 5'-CTTCTCCAAC(T/G)TCAGTCTTCAA; amplicon 2, forward 5'-CTAAGAGGTGTGTAACCACTACC and

reverse 5'-CTTCGCAATGATAGGAAGAGCC (García-Varela and Nadler, 2005). A partial (661-bp) sequence of mitochondrial cox 1 was amplified using the forward primer 5'-AGTTCTAATCATAA(R)GATA-T(Y)GG and reverse 5'-TAAACTTCAGGGTGACCAAAAAATCA (Folmer et al., 1994).

PCR reactions (25 µl) consisted of 10 µM of each primer, 2.5 µl of 10× buffer, 2 mM MgCl₂, and 1 U of Taq DNA polymerase (Platinum Taq, Invitrogen Corporation, São Paulo, Brazil). PCR cycling parameters for rDNA amplifications included denaturation at 94 °C for 3 min, followed by 35 cycles of 94 °C for 1 min, annealing at 50–58 °C (optimized for each rDNA amplification) for 1 min, and extension at 72 °C for 1 min, followed by a post-amplification incubation at 72 °C for 7 min. PCR cycling conditions for the cox 1 amplifications included denaturation at 94 °C for 5 min, followed by 35 cycles of 94 °C for 1 min, annealing at 40 °C for 1 min, and extension at 72 °C for 1 min, followed by a post-amplification incubation at 72 °C for 5 min.

Each PCR product was purified using Millipore columns (Amicon, Billerica, Massachusetts). Purified products were cloned by ligation into pGEM-T vector (Promega, Madison, Wisconsin) and used to transform competent *Escherichia coli* (JM109). Positive clones were identified by blue/white selection, and clone (insert) size was confirmed by PCR of DNA extracts prepared from bacterial (clone) colonies. Liquid cultures for minipreps were grown in Luria broth containing 50 µg/ml ampicillin. Plasmids for DNA sequencing were prepared using commercial miniprep kits (QIAprep, QIAGEN, Valencia, California). Plasmids were sequenced for both DNA strands using universal (vector) and internal primers. Sequencing reactions were performed using ABI Big Dye (Applied Biosystems, Boston, Massachusetts) terminator sequencing chemistry, and reaction products were separated and detected using an ABI 3730 capillary DNA sequencer. Contigs were assembled and base-calling differences resolved using Codoncode Aligner version 3.0.1 (Codoncode Corporation, Dedham, Massachusetts). The cox 1 sequences from the 2 *S. dimorpha* individuals were identical; therefore, only 1 specimen was used to obtain sequences for nuclear SSU and LSU rDNA. The sequences have been deposited in the GenBank database (Table I). A database was built for 18 isolates representing 16 species of polymorphids (including 2

isolates of *S. hispida*, 1 from México, and 1 from the Baltic Sea) with information obtained from the GenBank (Table I).

Alignments

The SSU and LSU data sets were aligned separately using ProAlign version 0.5 (Loytynoja and Milinkovitch, 2003). For each alignment, a ProAlign guide tree was constructed using corrected (for multiple hits) pairwise distances; this guide tree was used to estimate the hidden Markov model parameters (δ and ϵ) for progressive multiple alignment. Program (Java) memory and bandwidth were increased as required to complete the alignment. The minimum posterior probability of sites was used as the criterion for detecting and removing unreliably aligned sequence. To reduce the likelihood of excluding correctly aligned sites, the filter threshold was set to 60% minimum posterior probability. For the SSU sequences, using ProAlign to detect and remove unreliably aligned sequence by their posterior probabilities excluded 205 of 1,746 sites. For the LSU data set, 632 of 2,974 sites were excluded based on posterior probability filtering. Thus, these combined rDNA data sets included 3,883 characters after removal of unreliably aligned sites. Sequences from the mitochondrial protein coding gene *cox 1* were 655 bp in all taxa. These nucleotide sequences were readily aligned based on their inferred (in silico-translated) *cox 1* protein sequences. The concatenated 3-gene data set included 4,538 characters (filtered rDNA alignments plus *cox 1* with 18 sequences).

Phylogenetic analyses

The filtered SSU and LSU rDNA alignments were combined with the *cox 1* alignment to form a concatenated data set. Maximum parsimony (MP) and maximum likelihood (ML) trees were inferred using PAUP* 4.0b10 (Swofford, 2002). For ML analyses, the Akaike Information Criterion was used to assess the fit of general time reversible (GTR) nucleotide substitution models for the concatenated data set (Rodríguez et al., 1990) as implemented using Modeltest version 3.0 (Posada and Crandall, 1998). The best-fit substitution model for the concatenated data set it was GTR + I + G. For phylogenetic analysis, this GTR model with invariable sites (+ I) and rate heterogeneity (+ G) (Yang, 1994) was used. Tree searches were performed using 100 (ML) and 1,000 (MP) random taxon addition heuristic searches with branch and bound searches, respectively. Clade support was assessed by bootstrap resampling with 1,000 (ML) or 10,000 (MP) bootstrap replicates. MrBayes version 3.0b4 (Huelsenbeck and Ronquist, 2001) was used to analyze the concatenated data set, sampling every 5,000 trees over 5,000,000 generations (burnin determined empirically). For this analysis, a character partition corresponding to the 3 loci (nuclear SSU, LSU, and mitochondrial *cox 1*) was invoked. To each partition the best substitution model was GTR + I + G (Table II). The priors for the proportion of invariable sites (pinvarpr) were fixed separately for each partition with the values estimated by Modeltest (pinvarpr = 0.4044 for SSU, pinvarpr = 0.3893 for LSU, and pinvarpr = 0.3411 for *cox 1*).

To compare trees representing specific alternative phylogenetic hypotheses, topological constraints were defined on trees obtained from MP and ML analyses of the concatenated (SSU + LSU + *cox 1*) data set. Differences between unconstrained (best) and constrained trees representing alternative hypotheses were evaluated using the Shimodaira and Hasegawa likelihood test (Shimodaira and Hasegawa, 1999) and Templeton's (1983) modified parsimony test as executed in PAUP*. Trees were drawn using RETREE and DRAWGRAM from PHYLIP (Felsenstein, 1999). The observed (uncorrected) genetic differentiation between taxa was represented using the p-distance on the filtered concatenated dataset. The filtered concatenated alignment has been deposited in TreeBASE (Sanderson et al., 1994).

RESULTS

The concatenated data set of 3 genes (SSU + LSU + *cox 1*) included 18 taxa, with 4,538 characters, of which 900 were parsimony informative. The branch and bound search resulted in a single tree, with a consistency index (C.I.) = 0.594 and a length of 3,534 steps (Fig. 1). The tree indicates that Polymorphidae is a monophyletic group composed of 5 main clades. However,

TABLE II. Tree statistics for SSU, LSU, combined (SSU + LSU), *cox 1*, and concatenated (SSU + LSU + *cox 1*) data set. Number of informative characters, C.I., and tree length refer to parsimony inference. Pinv (proportion of invariable sites), Gd (shape of gamma distribution), -ln likelihood refer to maximum likelihood inference and Akaike Information Criterion (AIC) model inferred with Modeltest program.

Data set	Total characters	Uninformative characters	Constant characters	Informative characters	C.I.	Tree length	-ln likelihood	Pinv	Gd	Model AIC
SSU	1,541	235	1140	116	0.7555	634	5411.92192	0.4044	0.6553	GTR + I + G
LSU	2,342	313	1,614	415	0.7059	1,394	9877.23428	0.3893	0.5460	GTR + I + G
SSU + LSU	3,883	548	2574	581	0.7203	2,031	15368.65241	0.4069	0.5805	GTR + I + G
<i>cox1</i>	655	48	288	319	0.4282	1,490	6221.24492	0.3411	0.6086	GTR + I + G
SSU + LSU + <i>cox 1</i>	4,538	596	3,042	900	0.594	3,534	22400.47487	0.4493	0.4914	GTR + I + G

FIGURE 1. MP tree (3,534 steps) inferred from the concatenated SSU + LSU + cox 1 data set. Numbers above internal nodes show MP bootstrap clade frequencies.

Polymorphus and *Southwellina* are both paraphyletic. *Southwellina dimorpha* were included as sister taxa to *H. corynosoma*; this clade had 100% bootstrap support. The 2 isolates of *S. hispida* appear as sister taxa to *Polymorphus brevis* Van Cleave, 1916 and also had 100% bootstrap support (Fig. 1). The ML analysis yielded a single tree with $-\ln = 22400.4748$. The ML topology also yielded same main 5 clades as in the MP tree, but relationships among the clades were sometimes different for the position of *Pseudocorynosoma* (Fig. 2). The position of *S. dimorpha* with respect to *H. corynosoma* and the position of *S. hispida* with respect to *P. brevis* were also consistent between MP and ML analyses and are supported by high bootstrap values. To examine the separate contribution of each data set to the systematic position of the 2 species of *Southwellina*, additional phylogenetic analyses were conducted using SSU, LSU, and cox 1 alone and with the combined (SSU + LSU) data set (Table II). In addition, a Bayesian analysis was inferred with the concatenated (SSU + LSU + cox 1) data set. Three partitions were performed, and each partition corresponded with each gene. The Bayesian tree also supported the paraphyly of *Southwellina*. All ML and MP trees derived from individual and combined analyses as well

as the Bayesian inference supported the paraphyly of *Southwellina* and the sister relationship of *H. corynosoma* with *S. dimorpha* (trees not shown).

DESCRIPTION

Ibirhynchus gen. nov.

Diagnosis (based on 3 gravid females and 6 males): Trunk cylindrical, swollen in anterior region and slender posteriorly. Proboscis short, barrel-shaped, with 17 to 24 longitudinal rows of 9 to 14 hooks each. Sexual dimorphism in body size with females larger than males. Females possessing single field of spines in anterior region of trunk and males with 2 fields of spines. Neck long, proboscis receptacle double-walled. Lemnisci flat, broad, about same length, shorter than proboscis receptacle, cylindrical in shape. Male with 2 testes in tandem, located in anterior part of trunk, with 4 tubular cement glands (Figs. 3A–F; 4A, B).

Taxonomic summary

Type species: *Ibirhynchus dimorpha* (Schmidt, 1973) n. comb.

Host: White ibis, *Eudocimus albus* (L.), and whooping crane, *Grus americana* (L.) (definitive hosts); crayfish, *Procambarus clarkii* (Girard); and crawfish, *Cambarellus shufeldtii* (Faxon) (intermediate hosts).

Geographic distribution: Gulf of Mexico.

FIGURE 2. ML tree ($-\ln$ likelihood 22400.4748) inferred from the concatenated SSU + LSU + cox 1 data set. Numbers above internal nodes show ML bootstrap clade frequencies.

Remarks

The erection of the new genus to accommodate *S. dimorpha* is based on different data sources, i.e., the use of 3 molecular markers (2 nuclear and 1 mitochondrial) from which the phylogenetic position of the new genus, as well as levels of genetic divergence, were obtained. In addition, a detailed morphological comparison was made using both light and electron microscopy; some ecological information (host preference) also was considered. The new genus (*Ibirhynchus* gen. nov.) possesses a set of morphological characters that sets it apart from all the other congeners within Polymorphidae, i.e., the presence of a single field of spines in the anterior region of the trunk in females, 2 fields of spines in the region anterior of trunk in males, the presence of 4

tubular cement glands in males, and a barrel-shaped proboscis. In addition, sexual dimorphism in terms of spine distribution in the anterior region of the trunk is found only in the new genus (and in its only species, *I. dimorpha*); this trait represents an autapomorphy for the genus.

Our specimens, 6 males and 3 females collected from the type host white ibis from Veracruz state, México, were determined as *S. dimorpha* based on morphological criteria, following the description of this species provided by Schmidt (1973). To corroborate our identification, specimens of *S. dimorpha* were requested from HWML (vouchers 34731, 34732) and studied. *Southwellina dimorpha* is diagnosed by having a cylindrical trunk, swollen in the anterior region and slender in the posterior region, and a short and barrel-shaped proboscis possessing 17 to 24 longitudinal rows of

FIGURE 3. Line drawings of *Ibirhynchus dimorpha* n. gen., n. comb. from *Eudocimus albus*, Los Chivos, Veracruz state, México. (A) Adult male. (B) Adult female. (C) Proboscis female. (D) Anterior region of the trunk of female. (E) Proboscis of male. (F) Anterior region of trunk of male. Line drawings of *Southwellina hispida* from *Tigrisoma mexicanum*, Tlacotalpan, Veracruz, México. (G) Adult male. (H) Adult female. (I) Proboscis of male. (J) Anterior region of trunk of male. Bars = 1 mm (A–J).

FIGURE 4. Scanning electron micrographs of proboscis and trunk of adults of *Ibirhynchus dimorpha* n. gen., n. comb. (A) Male. (B) Female. Scanning electron micrographs of proboscis and trunk of adults of *Southwellina hispida*. (C) Male. (D) Female. Bars = 1 mm (A–C) and 400 μ m (D).

hooks with 9 to 14 hooks each. Females possess a single field of spines in the anterior region of the trunk and males have 2 fields of spines. Males possess 2 testes located in tandem in the anterior part of the trunk, with 4 tubular cement glands.

The molecular phylogenetic analyses showed that *Southwellina* is a paraphyletic genus and that *S. dimorpha* is closely related to *H. corynosoma*. Here, we propose the erection of a new genus to accommodate *S. dimorpha* and argue that it cannot be considered as a species of *Hexaglandula* because it possesses several morphological and ecological traits that guarantee its validity. A morphological synapomorphy for *Hexaglandula* is the presence of 6 cement glands in males, whereas the new genus possesses 4 cement glands. In addition, the new genus exhibits sexual dimorphism in the number of field of spines in the anterior portion of trunk (which represents an autapomorphy for the new genus). Furthermore, the new genus seems to be specific for the white ibis, whereas all the species of *Hexaglandula* are only found in typically fish-eating birds (herons). These characters (number of cement glands, fields of spines in the anterior portion of trunk, and host preference) are very important in the taxonomy of polymorphids and represent key characters for the distinction and classification of the entire family (Schmidt, 1973).

Currently, *Ibirhynchus* gen. nov. is monotypic, including the species *S. dimorpha* that was transferred to this genus as a new combination. As a result of this taxonomic change, the generic diagnosis of *Southwellina* had to be slightly emended to include solely species with 2 fields of spines in the anterior region of the trunk in both sexes, and with a modification in the number of longitudinal rows of spines in the proboscis and the number of hooks in each row.

REDESCRIPTION

Southwellina Witenberg, 1932

Diagnosis: Trunk cylindrical, swollen in anterior region and slender in posterior region. Both sexes contain 2 fields of spines in anterior region of trunk. Proboscis subcylindrical, slightly swollen in middle region and slender in anterior and posterior region, covered with 16 to 20 longitudinal rows of 12 to 17 hooks each, proboscis receptacle double-walled. Lemnisci longer than proboscis receptacle, cylindrical in shape. Females larger than males. Male possessing 2 oval-shaped testes contiguous or slightly tilted located in anterior portion of trunk, with 4 tubular cement glands (Figs. 3G–J; 4C, D).

Taxonomic summary

Type species: *Southwellina hispida* Van Cleave 1925.

Other species: *Southwellina macracanthus*.

Host: Fish-eating birds (herons, cormorants, grebes, pelicans and eagles) (definitive hosts); amphibians, reptiles, teleosts (paratenic); crayfish (intermediate hosts).

Geographic distribution: Cosmopolitan.

Remarks

The 38 mature adults of *S. hispida* collected in México were used for the morphological study and a comparison with specimens of *S. hispida* used by Schmidt (1973) in his revision of *Southwellina*. Voucher specimens of *S. hispida* (HWML) (accessions 34898, 34897, 34902, 34903) were used for comparison with our specimens. Based on the examination of these specimens, we concluded and confirmed that our specimens belong to *S. hispida* by possessing 2 fields of spines in the anterior region of the trunk in both sexes and males possessing 4 tubular cement glands. With respect to *S. macracanthus*, we also examined specimens from the HWML (vouchers 34527 and 34528) and confirmed that this species possesses the diagnostic characters of *Southwellina*, even though the species description is based solely on cystacanths.

DISCUSSION

The MP, ML, and Bayesian analyses inferred from the concatenated dataset of 3 genes showed that Polymorphidae is a monophyletic group; this result is strongly supported by bootstrap resampling, Bayesian posterior probabilities (BPP), and corroborated by findings of previous phylogenetic studies (see García-Varela and Pérez Ponce de León, 2008; García-Varela et al., 2009). The MP and ML trees also confirmed that *Polymorphus* (*P. brevis* and *P. minutus*) and *Southwellina* (*S. hispida* and *S. dimorpha*) are paraphyletic, suggesting that both genera represent species that should be re-examined and reclassified using morphological, ecological, and molecular data. In the present study, we included 2 of the 3 species of *Southwellina*.

Southwellina hispida was originally described as *Arhythmorhynchus hispida* based on cystacanths found in a frog from Japan (Van Cleave, 1925). Later, adults of *A. hispida* were described from a heron (*Nycticorax nycticorax*) (Fukui, 1929). Witenberg (1932) revised the diagnosis of *A. hispida* and elevated it to generic rank with the name *Southwellina*, but Yamaguti (1963) synonymized it with *Arhythmorhynchus*, which was accepted by other authorities (Van Cleave, 1945; Golvan, 1956). The diagnostic morphologic differences between *Southwellina* and *Arhythmorhynchus* include the presence of 1 or 2 fields of spines in the anterior region of the trunk in at least 1 sex, trunk length, proboscis shape, and number of cement glands in males. Based on these morphological features, Schmidt (1973) recognized the validity of the genus *Southwellina* and proposed that it was composed of 3 species (*S. hispida*, *S. dimorpha*, and *S. macracanthus*).

TABLE III. Comparative features among *Southwellina*, *Hexaglandula*, and *Ibirhynchus* gen. nov.

Character	<i>Southwellina</i>	<i>Hexaglandula</i>	<i>Ibirhynchus</i>
Trunk	Long and slender	Short and compact	Long and slender
Spines in the trunk	2 fields of spines on the anterior region on both sexes	Single field of spines on the anterior region on both sexes	Single field of spines on the anterior region in females and 2 fields of spines on males
Proboscis	Subcylindrical, slightly swollen in the middle region and slender on the anterior and posterior region	Subcylindrical, slightly swollen in the middle region and slender on the anterior and posterior region	Short, barrel-shaped
Proboscis hooks	Covered with 16 to 20 longitudinal rows with 12 to 17 hooks each	Covered with 16 longitudinal rows with 11 hooks each	Covered with 17 to 24 longitudinal rows with 9 to 14 hooks each
Cement glands	4 tubular	6 tubular	4 tubular
Adult host	Hérons, cormorants, grebes, pelicans, and eagles	Yellow crowned night heron (<i>Nyctanassa violacea</i>)	White ibis (<i>Eudocimus albus</i>)

In the present study, we used sequences of 2 isolates of the type species (*S. hispida*) collected from a wide geographic range (México and Denmark) and specimens of *S. dimorpha* collected from the type host (white ibis) from Veracruz state, México. The tree topologies derived from MP and ML analyses with a concatenated dataset of 3 genes placed the 2 isolates of *S. hispida* and *S. dimorpha* in 2 separate clades with strong bootstrap support. This result conflicts with their placement as congeners within *Southwellina* that is based on morphologic grounds. However, to test the paraphyly of *Southwellina*, the alternative hypothesis (*S. hispida* and *S. dimorpha* as a monophyletic group) was evaluated with MP and ML analyses using the concatenated data set of 3 genes. The constrained analyses of maximum parsimony and maximum likelihood showed a tree with a length of 3,756 steps and $-\ln = 22823.5321$, respectively, whereas original hypotheses of MP and ML yielded a tree with a length of 3,534 steps and $-\ln = 22400.4748$, respectively. Based on both Templeton's (1983) parsimony test and the Shimodaira and Hasegawa (1999) likelihood test, the alternative hypotheses of *S. hispida* and *S. dimorpha* as monophyletic is significantly worse than the best trees represented in Figures 1 and 2. The genetic divergence between the 2 isolates of *S. hispida* and *S. dimorpha* ranged from 10.7 to 11%; this divergence value is even higher compared with values obtained between congeneric species for other polymorphids in this phylogenetic analysis, i.e., *Corynosoma* (0.7–1.9%), *Profilicollis* (4.5–4.7%), and *Pseudocorynosoma* (2.0%). The phylogenetic position of *S. hispida* and *S. dimorpha* within Polymorphidae, their high level of genetic divergence, and morphologic differences (proboscis shape, and the presence of 1 or 2 fields of spines on the anterior region of the trunk in females; see Table III) support recognition of these separate evolutionary lineages as independent genera. *Southwellina hispida*, the type species, is retained in *Southwellina* with *S. macracanthus*. Both species are restricted to fish-eating birds (eagles, cormorants, grebes, pelicans, and herons). A new genus, *Ibirhynchus* gen. nov., is designated for *S. dimorpha* (see Remarks above).

Our phylogenetic hypotheses, based on the concatenated dataset of 3 genes plus the morphologic and ecological evidence, revealed the presence of a new genus, *Ibirhynchus*, within Polymorphidae. Our analyses also confirm that *Polymorphus* is paraphyletic, suggesting that the genus represents a complex of species that should be reexamined and reclassified using morphological, ecological, and molecular data. Therefore, the

inclusion of more congeneric species of *Polymorphus* and of other genera such as *Bolbosoma*, *Arythmorhynchus*, and *Diplospinifer* will be needed to produce a robust classification scheme, with the aim of better understanding of the evolutionary history of this enigmatic group of acanthocephalans.

ACKNOWLEDGMENTS

We are grateful to Berenit Mendoza-Garfias for help with the scanning electron microscopy, and Patricia de la Torre and Laura Marquez for help with the use of the DNA sequencer. This research was supported by the Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica (PAPIIT 215709) and the Consejo Nacional de Ciencia y Tecnología (CONACYT 102062) to MG-V and by the project CGL2007-63221 of the Ministry of Education and Science (MEC) of Spain to FJA and MG-V.

LITERATURE CITED

- AMERICAN ORNITHOLOGISTS' UNION. 1998. Check-list of North American birds, 7th ed. Washington, D.C., 829 p.
- AMIN, O. M. 1985. Classification. In *Biology of the Acanthocephala*, B. B. Nickol, and D. W. T. Crompton (eds.). Cambridge University Press, Cambridge, U.K., p. 27–72.
- AZNAR, F. J., G. PÉREZ PONCE DE LEÓN, AND J. A. RAGA. 2006. Status of *Corynosoma* (Acanthocephala: Polymorphidae) based on anatomical, ecological and phylogenetic evidence, with the erection of *Pseudocorynosoma* n. gen. *Journal of Parasitology* 92: 548–564.
- COURTNEY, C. H., AND D. J. FORRESTER. 1974. Helminth parasites of the brown pelican in Florida and Louisiana. *Journal of the Helminthological Society of Washington* 41: 89–93.
- DEZFULI, B. S., S. VOLPONI, I. BELTRAMI, AND R. POULIN. 2002. Intra- and interspecific density-dependent effects on growth in helminth parasites of the cormorant, *Phalacrocorax carbo sinensis*. *Parasitology* 124: 537–544.
- DIMITROVA, Z. M., AND B. B. GEORGIEV. 1994. *Ardeirhynchus* n. gen. (Palaeacanthocephala: Polymorphida: Polymorphidae), with a redescription of *A. spiralis* (Rudolphi, 1809) n. comb. *Systematic Parasitology* 29: 149–158.
- FELSENSTEIN, J. 1999. PHYLIP (phylogeny inference package), version 3.572. University of Washington, Seattle, Washington.
- FOLMER, O., M. BLACK, W. HOEH, R. LUTZ, AND R. VRIJENHOEK. 1994. DNA primers for the amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3: 294–299.
- FONT, W. F. 2007. Parasites of Hawaiian stream fishes: Sources and impacts. *Bishop Museum Bulletin in Cultural and Environmental Studies* 3: 157–169.
- FUKUI, T. 1929. On some acanthocephala found in Japan. *Annoto Zoology Japan* 12: 255–270.
- GARCÍA-PRIETO, L., M. GARCÍA-VARELA, B. MENDOZA-GARFÍAS, AND G. PÉREZ-PONCE DE LEÓN. 2010. Checklist of the Acanthocephala in wildlife vertebrates of México. *Zootaxa* 2419: 1–50.

- GARCÍA-VARELA, M., AND S. A. NADLER. 2005. Phylogenetic relationships of palaeacanthocephala (Acanthocephala) inferred from SSU and LSU rDNA gene sequences. *Journal of Parasitology* **91**: 1401–1409.
- , AND G. PÉREZ-PONCE DE LEÓN. 2008. Validating the systematic position of *Profilicollis* Meyer, 1931 and *Hexaglandula* Petrochenko, 1950 (Acanthocephala: Polymorphidae) using cytochrome c oxidase (cox 1). *Journal of Parasitology* **94**: 212–217.
- , F. J. AZNAR, AND S. A. NADLER. 2009. Systematic position of *Pseudocorynosoma* and *Andracantha* (Acanthocephala, Polymorphidae) based on nuclear and mitochondrial gene sequences. *Journal of Parasitology* **95**: 178–185.
- GOLVAN, Y. J. 1956. Acanthocéphales d'oiseaux. Première note. Description d' *Arhythmorhynchus longicollis* (Villot 1875) et revision du genre *Arhythmorhynchus* Lühe 1911 (Acanthocephala). *Annales de Parasitologie Humaine et Comparée* **31**: 199–224.
- GUILLÉN-HERNÁNDEZ, S., M. GARCÍA-VARELA, AND G. PÉREZ-PONCE DE LEÓN. 2008. First record of *Hexaglandula corynosoma* (Travassos, 1915) Petrochenko, 1958 (Acanthocephala: Polymorphidae) in intermediate and definitive hosts in México. *Zootaxa* **1873**: 61–68.
- HOBERG, E. P. 1986. Aspects of ecology and biogeography of Acanthocephala in Antarctic seabirds. *Annales de Parasitologie Humaine et Comparée* **61**: 199–214.
- HOWELL, S. N. G., AND S. WEBB. 1995. A guide to the birds of México and Northern Central America. Oxford University Press, New York, New York, 851 p.
- HUELSENBECK, J. P., AND F. RONQUIST. 2001. MrBayes: Bayesian inference of phylogeny. Version 3.0b4. Distributed by the author. Department of Biology, University of Rochester, Rochester, New York.
- LOYTYNOJA, A., AND M. C. MILINKOVITCH. 2003. A hidden Markov model for progressive multiple alignment. *Bioinformatics* **19**: 1505–1513.
- NICKOL, B. B., D. W. T. CROMPTON., AND D. W. SEARLE. 1999. Reintroduction of *Profilicollis* Meyer, 1931, as a genus in Acanthocephala: Significance of the intermediate host. *Journal of Parasitology* **85**: 716–718.
- , R. W. HEARD, AND N. F. SMITH. 2002. Acanthocephalans from crabs in the southeastern US, with the first intermediate hosts known for *Arhythmorhynchus frassoni* and *Hexaglandula corynosoma*. *Journal of Parasitology* **88**: 79–83.
- NORMAN, O. D., C. K. BLEND, AND C. K. ANDERSON. 2003. Endohelminths from the brown pelican, *Pelecanus occidentalis*, and the American white pelican, *Pelecanus erythrorhynchus*, from Galveston Bay, Texas, U.S.A., and checklist of pelican parasites. *Comparative Parasitology* **70**: 140–154.
- PETROCHENKO, V. I. 1958. Acanthocephala of domestic and wild animals, vol. II. Izdatel'stvo Akademii Nauk SSSR, Vsesoyuznoe Obshchestvo Gel'mintologov, Moscow, Russia, 478 p.
- POSADA, D., AND K. A. CRANDALL. 1998. Modeltest: Testing the model of DNA substitution. *Bioinformatics* **9**: 817–818.
- RICHARDSON, J. D., AND R. A. COLE. 1997. Acanthocephala of the bald eagle (*Haliaeetus leucocephalus*) in North America. *Journal of Parasitology* **83**: 540–541.
- , AND W. F. FONT. 2006. The Cajun dwarf crawfish (*Cambellus shufeldtii*): An intermediate host for *Southwellina dimorpha* (Acanthocephala). *Journal of the Arkansas Academy of Science* **60**: 192–193.
- RODRÍGUEZ, F., J. F. OLIVER, A. MARIN, AND J. R. MEDINA. 1990. The general stochastic model of nucleotide substitution. *Journal of Theoretical Biology* **142**: 817–818.
- SANDERSON, M. J., M. J. DONOGHUE, W. PIEL, AND T. ERIKSSON. 1994. TreeBASE: A prototype database of phylogeny analyses and an interactive tool for browsing the phylogeny of life. *American Journal of Botany* **81**: 183.
- SCHMIDT, G. D. 1973. Resurrection of *Southwellina* Witenberg, 1932, with a description of *Southwellina dimorpha* sp. n., a key to genera in Polymorphida (Acanthocephala). *Journal of Parasitology* **59**: 299–305.
- . 1985. Development and life cycles. In *Biology of Acanthocephala*, B. B. Nickol and D. W. T. Crompton (eds.). Cambridge University Press, Cambridge, U.K., p. 273–286.
- SHIMODAIRA, H., AND M. HASAGAWA. 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. *Molecular Biology and Evolution* **16**: 1114–1116.
- SWOFFORD, D. L. 2002. PAUP 4.0b10. Phylogenetic analysis using parsimony (and other methods). Sinauer Associates, Sunderland, Massachusetts.
- TEMPLETON, A. R. 1983. Phylogenetic inference from restriction endonuclease cleavage site maps with particular reference to the humans and apes. *Evolution* **37**: 221–224.
- VAN CLEAVE, H. J. 1925. Acanthocephala from Japan. *Parasitology* **17**: 149–156.
- . 1945. The status of the acanthocephalan genus *Arhythmorhynchus*, with particular references to the validity of *A. brevis*. *Transactions of American Microscopical Society* **64**: 133–137.
- WARD, H. L., AND H. A. WINTER. 1952. Juvenile Acanthocephala from the yellowfin croaker, *Umbrina roncadore*, with description of a new species of the genus *Arhythmorhynchus*. *Transactions of American Microscopical Society* **71**: 154–156.
- WITENBERG, G. 1932. Acanthocephalen-studien II. Ueber das system der Acanthocephalen. *Bolletín Zoology Napoli* **3**: 253–266.
- YAMAGUTI, S. 1963. *Systema helminthum*, vol. V. Acanthocephala. Interscience Publishers, New York, 423 p.
- YANG, Z. 1994. Estimating the patterns of nucleotide substitution. *Journal of Molecular Evolution* **39**: 105–111.