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

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

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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 6 genera (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).

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).
Six adult acanthocephalans identified as S. dimorpha and S. hispida were prepared for scanning electron microscopy (SEM) using standard methods (Güllen-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 (including 2

### 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.

<table>
<thead>
<tr>
<th>Species</th>
<th>Host</th>
<th>Locality</th>
<th>Vouchers (CNHE)</th>
<th>GenBank accession Cox</th>
<th>GenBank accession SSU</th>
<th>GenBank accession LSU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Andracantha gravida</td>
<td>Phalacrocorax auritus</td>
<td>Yucatán, México</td>
<td>5997</td>
<td>EU267822</td>
<td>EU267802</td>
<td>EU267814</td>
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<td>Corynosoma enhydri</td>
<td>Enhydra lutris</td>
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<td>3429</td>
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<td>AF011837</td>
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<td>Corynosoma magdaleni</td>
<td>Phoca hispida sainsensis</td>
<td>Lake Saimaa, Finland</td>
<td>EF467872</td>
<td>EU267803</td>
<td>EU267815</td>
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<tr>
<td>Corynosoma strammosum</td>
<td>Phoca vitulina</td>
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<td>EF467870</td>
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<td>EU267816</td>
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<tr>
<td>Tibirhynchus dimorpha</td>
<td>Eudocimus albus</td>
<td>Veracruz, México</td>
<td>6164</td>
<td>GQ981438*</td>
<td>GQ981436*</td>
<td>GQ981437*</td>
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<td>Hexaglandula coronoidea</td>
<td>Nyctanassa violacea</td>
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<td>5765</td>
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<td>EU267817</td>
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<tr>
<td>Polynemus brevis</td>
<td>Nycticorax nycticorax</td>
<td>Michoacán, México</td>
<td>5777</td>
<td>DQ089717</td>
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<td>Polymorphus minutus</td>
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<td>Dijon, France</td>
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<td>EU267806</td>
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<td>Profillicollis botulus1</td>
<td>Enhydra lutris</td>
<td>Monterey Bay, California, USA</td>
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<td>DQ089720</td>
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<tr>
<td>Profillicollis botulus2</td>
<td>Somateria mollissima</td>
<td>Denmark</td>
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<td>EF467862</td>
<td>EU267805</td>
<td>EU267818</td>
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<td>Pseudocorynosoma constrictum</td>
<td>Anas platyrhynchos</td>
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<td>Bucephala rolea</td>
<td>Durango, México</td>
<td>5720</td>
<td>EU267820</td>
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<td></td>
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<tr>
<td>Southwellina hispida2</td>
<td>ND</td>
<td>Baltic Sea, Finland</td>
<td>EF467866</td>
<td>EU267809</td>
<td>EU267810</td>
<td></td>
</tr>
<tr>
<td>Southwellina hispida2</td>
<td>Tigrisoma mexicanum</td>
<td>Veracruz, México</td>
<td>EF467867</td>
<td>EU267807</td>
<td>EU267811</td>
<td></td>
</tr>
<tr>
<td>Centrorhynchus sp.</td>
<td>Falco peregrinus</td>
<td>California, USA</td>
<td>DQ089716</td>
<td>AY830155</td>
<td>AY829104</td>
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</tr>
<tr>
<td>Gorgorhynchoidea bullocki</td>
<td>Eugerres plumieri</td>
<td>Quintana Roo, México</td>
<td>DQ089715</td>
<td>AY830154</td>
<td>AY829103</td>
<td></td>
</tr>
<tr>
<td>Plagiorhynchus cylindraceus</td>
<td>Porcilio saber</td>
<td>Dijon, France</td>
<td>DQ089724</td>
<td>AF001839</td>
<td>AY829102</td>
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</tr>
</tbody>
</table>

### 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'-AGTTAAAGCCATGCTAGCAGT-3' and reverse primer 5'-GCAGGTT-CACCTACGGAAA-3'. 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'-CAAGTACCGTGAGGAAATGTTGC-3' and reverse 5'-CTTCTTCACAT(T)GTCAGCTTCAA-3', and forward 5'-CTAAGGAGTGTGTAACAACTACC-3'. Positive clones were identified by sequencing DNA extracts prepared from bacterial (clone) colonies. Liquid cultures for minipreps were grown in Luria broth containing 50 μg/ml ampicillin. 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 (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 (θ 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 (Rodriguez et al., 1990) as implemented using Modeltest version 3.0 (Posada and Crandall, 1998). The best-fit substitution model for the concatenated data set was GTR + I + G. For phylogenetic analysis, this GTR model with invariant 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 = 0.4099 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,

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**Table II.** Tree statistics for SSU, LSU, and concatenated (SSU + LSU + cox 1) dataset. Number of informative characters, C.I., and tree length refer to parsimony inference. Pin (proportion of invariable sites), Gd (shape of gamma distribution), ln likelihood refer to maximum likelihood inference and AIC model inferred with Modeltest program.

<table>
<thead>
<tr>
<th>Data set</th>
<th>Informative characters</th>
<th>Constant characters</th>
<th>Total characters</th>
<th>Uninformative characters</th>
<th>C.I.</th>
<th>Tree length</th>
<th>ln likelihood</th>
<th>Pinvarpr (%)</th>
<th>Gd</th>
<th>Model AIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSU</td>
<td>1,541</td>
<td>235</td>
<td>1,776</td>
<td>235</td>
<td>1.16</td>
<td>1,490</td>
<td>5,434</td>
<td>0.6755</td>
<td>0.7555</td>
<td>GTR + I + G</td>
</tr>
<tr>
<td>LSU</td>
<td>1,440</td>
<td>313</td>
<td>1,753</td>
<td>313</td>
<td>0.7059</td>
<td>1,490</td>
<td>5,434</td>
<td>0.6560</td>
<td>0.5805</td>
<td>GTR + I + G</td>
</tr>
<tr>
<td>SSU + LSU</td>
<td>2,981</td>
<td>1,614</td>
<td>4,595</td>
<td>1,614</td>
<td>0.7016</td>
<td>2,974</td>
<td>10,240</td>
<td>0.4044</td>
<td>0.3411</td>
<td>GTR + I + G</td>
</tr>
<tr>
<td>SSU + LSU + cox1</td>
<td>4,538</td>
<td>655</td>
<td>5,193</td>
<td>655</td>
<td>0.7003</td>
<td>4,534</td>
<td>22,400</td>
<td>0.4044</td>
<td>0.3411</td>
<td>GTR + I + G</td>
</tr>
</tbody>
</table>

---
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 shufeldti* (Faxon) (intermediate hosts).

*Geographic distribution:* Gulf of Mexico.
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, Mexico, 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
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. Lemmisci 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.

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 Mexico 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 Arhythnorhynchus hispida based on cystacanths found in a frog from Japan (Van Cleave, 1925). Later, adults of A. hispida were described from a heron (Nycitcorax nictitorax) (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 Arhythnorhynchus, which was accepted by other authorities (Van Cleave, 1945; Golvan, 1956). The diagnostic morphologic differences between Southwellina and Arhythnorhynchus 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*, *Hexaglandulosa*, and *Ibirhynchus* gen. nov.

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

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 congensers 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 dataset 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*, *Artychomorhynchus*, and *Diplospifer* 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.

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LITERATURE CITED


