

EMPYREUMA SPECIES AND SPECIES LIMITS: EVIDENCE FROM MORPHOLOGY AND
MOLECULES (ARCTIIDAE: ARCTIINAE: CTENUCHINI)

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ABSTRACT. Species limits within *Empyreuma* are addressed using a morphological study of male and female genitalia and sequence data from the mitochondrial gene COI. Currently, four species are recognized: *E. pugione* (L.), *E. affinis* Rothschild, *E. heros* Bates, *E. anassa* Forbes. Two entities can be readily distinguished, the Jamaican *E. anassa* and a widespread *E. pugione*-complex, based on adult morphology. Neither *E. affinis* nor *E. heros* can be distinguished by coloration or genitalic differences. Analysis of COI haplotypes suggests that *E. affinis* is not genetically distinct from *E. pugione* (<1% sequence divergence); however, the population from the Bahamas, *E. heros*, is differentiated from other haplotypes with an uncorrected sequence divergence of 5%. We place *E. affinis* Rothschild, 1912 as a **new synonym** of *E. pugione* Hübner 1818, and recognize three species: *E. anassa*, *E. pugione*, and *E. heros*. This paper includes a revised synonymic checklist of species and a redescription of the genus, with notes on biology, and with illustrations of male genitalia, female genitalia, wing venation, and abdominal sclerites.

Additional key words: Caribbean fauna, Greater Antilles, mimicry, phylogeography, systematics.

The tiger moth genus *Empyreuma* Hübner (Arctiidae: Arctiinae: Ctenuchini) (Hübner 1818) is endemic to the Greater Antilles of the Caribbean, and has expanded its distribution into Florida (Adam & Goss 1978, Franclemont 1983). Adults are colorful mimics of the wasp *Pepsis rubra* Drury (Hymenoptera: Pompilidae) (Fig. 1A–D), and the host plant for all species reared to date is *Nerium oleander* (L.) (Apocynaceae). Mating behavior of *E. pugione* (L.) involves ultrasound signaling between males and females (Coro et al. 1983, Otaño et al. 1987, Perez et al. 1988, Portilla et al. 1987, Wilson 1999). In some of these studies, *E. pugione* was misidentified as *E. affinis* Rothschild. Continued confusion over the species status of *E. pugione*, *E. affinis*, and other members of this genus frustrates attempts to interpret mating experiments among populations obtained from different locations in the Caribbean.

Previous taxonomic treatments have been summarized in an annotated synonymic checklist by J. Donahue (unpublished). Currently, four valid species and two subspecies names are recognized in *Empyreuma*. These include *E. pugione* (type species; type locality Virgin Islands), *E. affinis affinis* Rothschild (type locality Cuba), *E. affinis haitiensis* Rothschild (type locality Haiti), *E. anassa* Forbes (type locality Jamaica), and *E. heros* Bates (type locality Bahamas). Forbes (1917:344) treated *E. affinis* and *E. pugione* as separate species when he described *E. anassa*, but later (Forbes 1930) refers to just two species, one restricted to Jamaica (*E. anassa*) and one widespread throughout the Greater Antilles. That is, Forbes considered *E. pugione* and *E. affinis* conspecific, although he did not formally place *E.*

affinis as a junior synonym of *E. pugione*. Bates (1934) subsequently described *E. heros* from the Bahamas, but he did not provide figures or diagnostic features that separate it from previously described species. Thus, the question remains whether either or both of these described species are junior synonyms of *E. pugione*.

We report here the results of a morphological survey of genitalia and a molecular characterization of haplotypes of *E. pugione*, *E. affinis*, and *E. heros*. We find that the morphological evidence supports recognition of two species, *E. anassa* and a widespread, externally variable *E. pugione* as Forbes (1930) suggested. In contrast, haplotype differentiation suggests that the population in the Bahamas is genetically distinct from other populations of *E. pugione* supporting recognition of *E. heros* as a third, valid species.

MATERIALS AND METHODS

Morphology. Standard genitalia dissections were done (Winter 2000). Abdomens were softened in warm 10% KOH for 5–15 minutes and then cleaned (scales and viscera removed) in several rinses of 40% ethanol. Abdominal sclerites and genitalia were stained with chlorazole black E (Sigma, St. Louis, MO) dissolved in deionized distilled water (saturated). Specimens were viewed in 40% ethanol. Wings were bleached, neutralized in weak acetic acid, rinsed and stained overnight in Eosin Y (1% in distilled water; Fisher Scientific, Pittsburgh, PA). Permanent slide mounts (Canada balsam, Sigma, St. Louis, MO) were made of abdominal pelts, genitalia and wings (Winter 2000).

Genital preparations of 18 reared individuals (9 males, 9 females) were examined to assess variation within a population (Table 1). These individuals were offspring of pairs of wild caught individuals (W. Conner pers. com.). Type specimens of *E. affinis affinis* Rothschild (BMNH), *E. sanguinea* Rothschild, *E. a. haitiensis* Rothschild (BMNH; 2 males and 2 female

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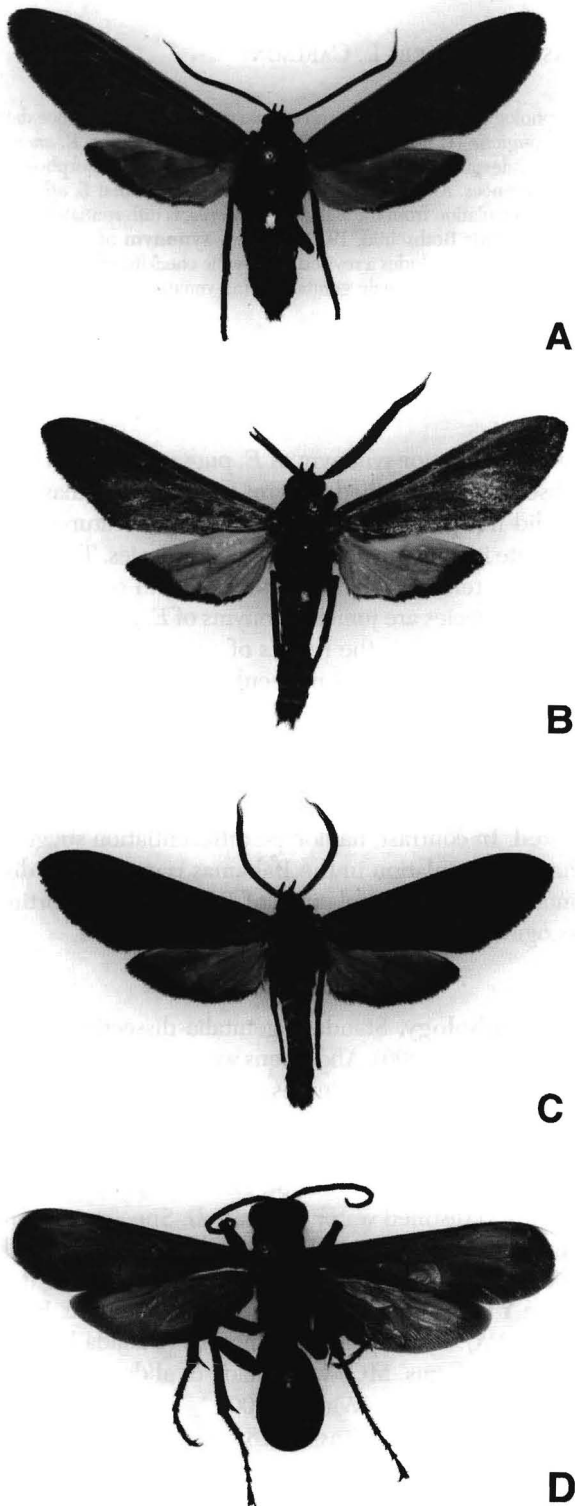


FIG. 1. Adult males of *Empyreuma pugione* L. (A), *E. heros* (B), and *E. anassa* Forbes (C); Adult female of *Pepsis rubra* Drury (Hymenoptera) (D).

syntypes including #2459), *E. a. portoricensis* Rothschild (female syntype 2460) were examined. The *E. heros* type was not available for loan from the MCZ and its image is not on-line on the type specimen database. The species *E. anassa* is distinct and the uncus sufficiently illustrated by Forbes (1917) to allow confident determination. Additional dissections of specimens were made that represented type localities of *E. anassa*, *E. affinis*, *E. heros*, and *E. pugione*. Camera lucida drawings were made of selected specimens. Specimen deposition and genitalic preparation numbers are indicated in "Specimens examined" and in Table 1. Wing measurements were taken from the center of the thorax to the wing tip (wing length) and from wing tip to wing tip (wing span).

Terminology for abdominal and genital morphology follows Klots (1970) and Forbes (1939). Collections consulted include: FSMC, Allyn Museum, Florida State University (J.Y. Miller); BMNH, The Natural History Museum, London (M. Scoble); MNHP, Muséum National d'Histoire Naturelle, Laboratoire d'Entomologie, Paris (J. Minet); NMNH, National Museum of Natural History, Smithsonian Institution, Washington, D.C. (M. Pogue); UMSP, University of Minnesota Saint Paul Insect Collection (R. Holzenthal).

Gene region and analysis. Thirteen individuals from the Puerto Rican colony were sequenced for the mitochondrial gene COI and these represent *E. pugione portoricensis* Rothschild. Eight individuals were collected from Fort Lauderdale (Florida) and represent *E. affinis*. Identifications were confirmed at the NMNH by R. Wilson and R. Simmons. These vouchers were deposited at the Insect Museum (St. Paul, Minnesota). Museum specimens were used to obtain a set of individuals (9) from the Bahamas, and legs of these were extracted to represent *E. heros*. Multiple attempts to extract DNA from museum specimens of *E. anassa* were unsuccessful. We suspect that traditional preparation techniques, drying in paper envelopes followed by relaxation for spreading, degraded the DNA. Museum specimens were collected over multiple years and a single leg per museum specimen was used. For each individual, the source colony or museum collection, voucher number, and sex are reported in Table 1.

DNA extractions were performed using the DNeasy Tissue Kit® (QIAGEN Inc., Santa Clarita, CA) and the Insect extraction protocol (DNeasy Tissue protocol 1997) with 20 µl of Proteinase K (20 mg/ml). Either frozen material (-20°C) or legs of museum specimens were used. Museum specimens were extracted on separate days from fresh material to minimize contamination with similar DNA. DNA extrac-

TABLE 1. Specimens used in mtDNA study. "SJW#" = genitalic preparation of individual, "DNA#" = DNA voucher number for same, E. = *Empyreuma*, — = not applicable.

Genus species	Voucher no. dissection	DNA	Source/label data	Haplotype number	GENBANK number	Voucher depository	
<i>E. pugione</i>	SJW1054♀	DNA900	Puerto Rico Colony	1	AF513059	UMSP	
	SJW1055♀	DNA901	Puerto Rico Colony	2	AF513060	UMSP	
	SJW1056♂	DNA903	Puerto Rico Colony	3	AF513062	UMSP	
	SJW1057♂	DNA904	Puerto Rico Colony	4	AF513063	UMSP	
	SJW1058♂	DNA905	Puerto Rico Colony	5	AF513064	UMSP	
	SJW1059♂	DNA906	Puerto Rico Colony	6	AF513065	UMSP	
	SJW1062♀	DNA909	Puerto Rico Colony	7	AF513068	UMSP	
	SJW1063♀	DNA910	Puerto Rico Colony	8	AF513069	UMSP	
	SJW1064♀	DNA911	Puerto Rico Colony	9	AF513070	UMSP	
	SJW1068♀	DNA918	Puerto Rico Colony	11	AF513075	UMSP	
	SJW1070♂	DNA920	Puerto Rico Colony	3	AF513077	UMSP	
	SJW 1080♂	DNA921	Puerto Rico Colony	—	—	UMSP	
	<i>E. affinis</i>	SJW1056♀	DNA902	Florida Colony	12	AF513061	UMSP
		SJW1060♂	DNA907	Florida Colony	13	AF513066	UMSP
		SJW1061♀	DNA908	Florida Colony	14	AF513067	UMSP
		SJW1065♂	DNA912	Florida Colony	15	AF513071	UMSP
		—	DNA913	Florida Colony	10	AF513072	UMSP
		SJW1066♂	DNA914	Florida Colony	16	AF513073	UMSP
		SJW1067♂	DNA917	Florida Colony	17	AF513074	UMSP
SJW1069♂		DNA919	Florida Colony	18	AF513076	UMSP	
—		DNA922	Florida Colony	19	AF513078	UMSP	
<i>E. heros</i>		SJW1081♂	DNAS043	Bahamas: Long Island	21	AF513083	FSMC
	SJW1082♂	DNAS038	Bahamas: Crooked Island	20	AF513080	FSMC	
	SJW1083♀	DNAS041	Bahamas: Crooked Island	21	AF513081	FSMC	
		DNAS042	Bahamas	22	AF513082	FSMC	
<i>Nyridela</i> sp.	—	DNA069	Las Alturas, Costa Rica	—	AF513079	UMSP	
<i>Scena potentia</i>	—	DNA008	Las Alturas, Costa Rica	—	AF277448	UMSP	

tion control blanks were maintained for each museum extraction set. These blanks were checked for volatile DNA contamination by including them in the PCR amplifications. All extraction blanks were negative (did not contain DNA) when used as template for PCR.

The entire COI gene was amplified using PCR (Saiki et al. 1988) for lab colony individuals (Table 1). The COI primers amplify nearly 1500 bp of COI. To amplify COI, five primers (two external, three internal) were used. The external primers were K698 (5'-TAC AAT TTA TCG CCT AAA CTT CAG CC-3') and PAT2K837 (5'-TCC ATT ACA TAT AAT CTG CCA TAT TAG-3') that have 5' ends located at positions 1436 and 3037, respectively, on the *Drosophila* mt genome (Clary & Wolstenholme 1985). Three internal primers were also used: C1-J-1751 (alias RON), C1-N-2191 (alias NANCY), and REVNANCY (5'-GAA GTT TAT ATT TTA ATT TTA CCG GG-3'; position at 5': 2190) (Simon et al. 1994).

Based on initial results of haplotype variation, only the more variable portion of COI, a 550 bp piece (revNancy-Pat2K837), was amplified and sequenced for specimens from the Bahamas (*E. heros*). For all reactions, a hot start (95°C dwell, 1 min) prior to addition of TAQ was used. Cycling parameters were: 29 cycles (94°C, 1 min, 45°C, 1 min, 72°C, 1 min), 1 cycle

(94°C, 1 min, 45°C, 1 min, 72°C, 10 min), 4°C for a minimum of 4 minutes. PCR products were cleaned for automated sequencing with a Qiaquick PCR purification kit® (QIAGEN Inc., Santa Clarita, CA) according to protocol. Sequencing reactions were performed using Bigdye terminator kit (PE Biosystems) using 10 µm of primer and 1–6 µl of clean PCR product. Sequencing reactions were performed using a BigDye Terminator Cycle Sequencing Ready Reaction Sequencing Kit® (PE Applied Biosystems, Foster City, CA). We performed half reactions and used 2 µL of 10 µM sequencing primer, 1–6 µL of clean PCR product, and 8–13 µL ddH₂O (final volume: 20 µL). Recommended sequencing cycling parameters were used. Each sample was cleaned using Sephadex columns (Centri-Sep protocol; Princeton Separations, Inc., Adelphia, NJ). Samples were then resuspended in 20 µL of Template Suppression Reagent (TSR)® (PE Applied Biosystems, Foster City, CA). An ABI 310 system was used to visualize and record the sequence. Typically, sequences up to 750 bp were obtained with the long capillary for COI.

Data were imported into Sequencher 3.1.1® (Gene Codes Corp., Ann Arbor MI). Sequences for each individual were aligned to produce a consensus sequence and the sequence translated and checked for

stop codons. Individuals were then aligned by conserved motifs and adjusted by eye when necessary.

Phylogenetic analysis. As the relationship of *Empyreuma* to other ctenuchines and euchromiines is unknown, we established potential outgroups by performing an initial analysis with one sequence of *Empyreuma* and all available ctenuchine and euchromiine species (Simmons & Weller 2001). We then selected species of two genera, *Nyridela* sp. and *Scena potentia* (Druce), to root the analysis of *Empyreuma* haplotypes. These taxa are given in Table 1 with their GENBANK accession numbers. Unique haplotypes were identified for the *Empyreuma* specimens. These were analyzed using heuristic searches and maximum parsimony (PAUP*) (Swofford 2000). All positions were equally weighted, and 10 random additions were performed to search for tree islands (Maddison 1991). The parsimony results were then used to generate likelihood parameters for a maximum likelihood analysis (ML) using the following menu options: Trees: Tree scores: Likelihood. The following likelihood parameters were used: HKY-85 model, transition-transversion ratio of two, and empirical nucleotide frequencies.

RESULTS AND DISCUSSION

How many species? Morphology clearly supports the recognition of two entities, *E. anassa*, *E. pugione*-complex, whereas molecular results supports three species, *E. anassa*, *E. pugione*, and *E. heros*. The male genitalia (Figs. 2–3) and female genitalia (Fig. 4) of *E. anassa* and the *E. pugione* species-complex are distinct. However, we could not identify consistent, adult morphological features to diagnose *E. heros* compared to *E. pugione* (compare Fig. 2B, C). Both coloration and armature of the male vesica lack fixed differences.

Our molecular results differentiate between *E. heros* (the Bahamas), and other *E. pugione* populations. The mtDNA sequences of *Empyreuma* were typical for COI in insects (Simmons & Weller 2001), with an A/T bias, especially pronounced in the third codon position (A = 43%, C = 12%, G = 2%, T = 43%). We obtained approximately 1474 bp of COI sequence for *E. pugione* and *E. affinis*, and 450 bp for *E. heros*, from approximately 2190 to 3037 (revNancy-Pat2K837). Of 1474 bp, 54 bp were informative (4%); the majority of this variation was third positions (first: 10/54, second: 5/54, third: 39/54). We obtained 19 distinct haplotypes for the combined sample of *E. pugione* and *E. affinis*; three were obtained for *E. heros*. The uncorrected p-distance between *E. heros* to the other haplotypes was 5%. There are 17 unique substitutions for *E. heros* (Table 2), and all are third position transitions.

The MP analysis of the COI data resulted in over 139,000 trees (length = 302, consistency index = 0.66, retention index = 0.69; trees not shown). The ML analysis (Fig. 5) has a $-\ln L = 3571.17$. Individuals from the Puerto Rico population (*E. pugione portoricensis*) and from the Florida population (*E. affinis*) do not segregate into two, reciprocally monophyletic taxa (Fig. 5). In contrast, the haplotypes from the Bahamas are recovered as a separate clade in all observed COI topologies. Cryptic species in leaf-mining flies and other insects have been identified by COI and other molecular markers (e.g., Frolich et al. 1999, Scheffer 2000, Scheffer & Lewis 2001). The genetic divergence between *E. affinis* and *E. pugione* (<1%) is slightly higher than divergences among races of *Heliconius erato* (0.5%; Brower 1994a, b) or agromyzid flies (0.6%; Scheffer & Weigmann 2000); however, there is no clustering pattern to the *pugione-affinis* haplotypes (Fig. 5). Similarly, a study of Western spruce budworm species' limits (Sperling & Hickey 1994) suggested that the designation of *Choristoneura biennis* Free., *C. orae* Free., and *C. occidentalis* Free. could not be supported because of low sequence divergence (<1%), and their haplotypes were placed in the same clade.

Both morphological and molecular results support placing *E. affinis* as a junior synonym of *E. pugione*. Molecular results support maintaining the species' status of the Bahaman population, *E. heros*. Additional sampling and molecular study is warranted to confirm these results; however, these initial findings support treating this population as a unique, endemic lineage that should be considered when forming conservation strategies for the Bahamas. Additionally, other islands in the Caribbean may harbor cryptic genetic diversity and greater sampling is needed.

Phylogenetic placement. The systematic placement of *Empyreuma* is unclear. Although a preliminary study of Ctenuchini and Euchromiini identified *Scena* and *Nyridela* as potential sister genera (Simmons & Weller 2001), that study was focused on assessing the monophyly of tribes, not identifying the nearest sister genus or genera to *Empyreuma*. Our COI results suggest that these genera, *Scena* and *Nyridela*, are not sister to *Empyreuma*. Rooting trees with *Scena* and *Nyridela* places the root at the midpoint of the longest branch: the branch connecting the *E. heros* clade to the *E. pugione* haplotypes (Fig. 5). That is, using *Scena* and *Nyridela* as outgroups was no better than arbitrarily selecting midpoint rooting for our analysis. Morphological data does not support a close relationship either. Males of *Scena potentia* Druce have a bifid uncus apically, but it is stalked at the base and probably not homologous with the bifid

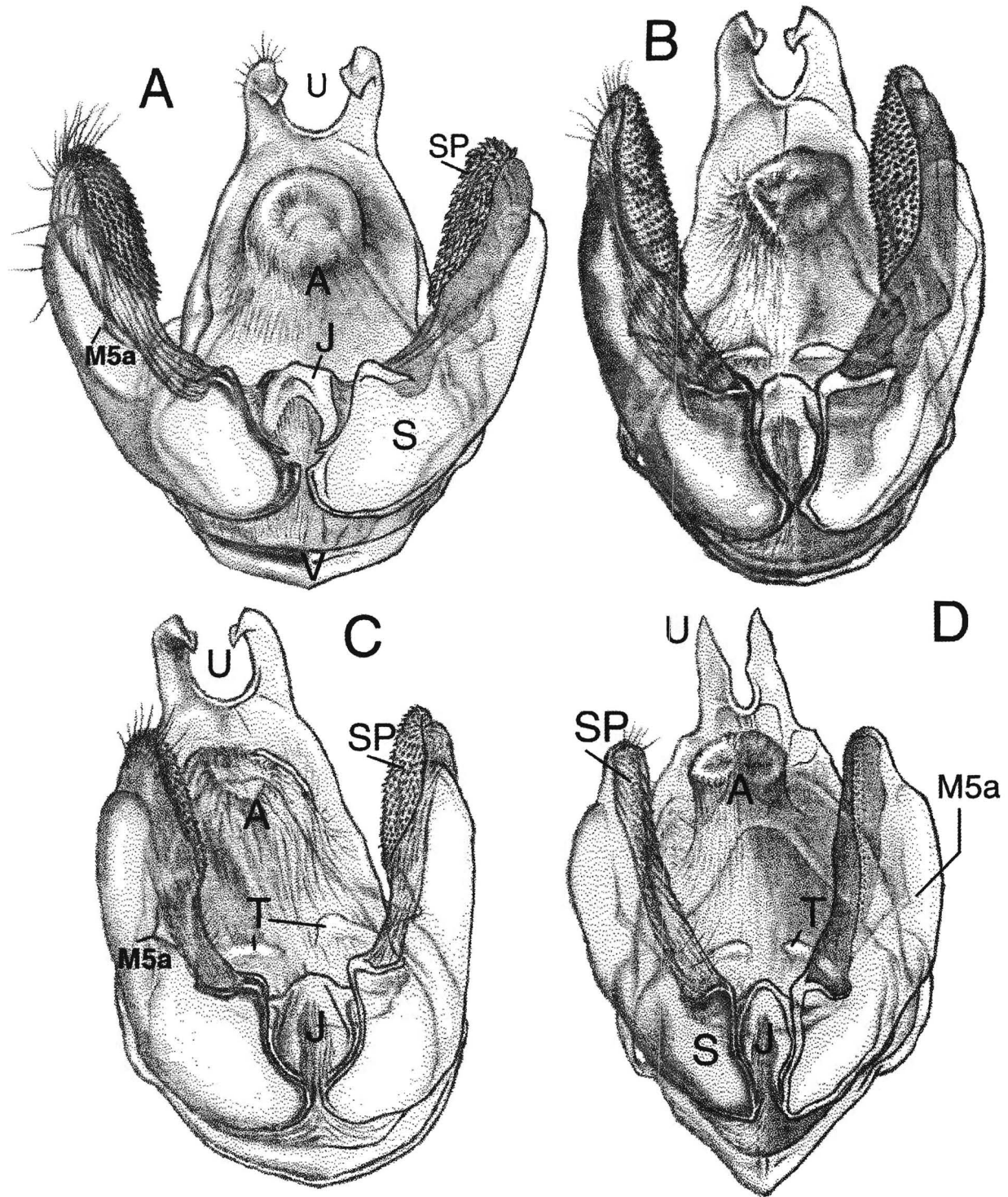


FIG. 2. Male genitalia of: *E. pugione* culture, SJW1066 (A), *E. pugione* Dom. Rep., SJW1005 (B), *E. heros*, SJW1001, (C), *E. anassa*, Jamaica, SJW999 (D). A = anal tube, J = juxta, M5a = muscle attachment process, S = sacculus, SP = spinose pad, T = transtilla, U = bifid uncus, V = vinculum.

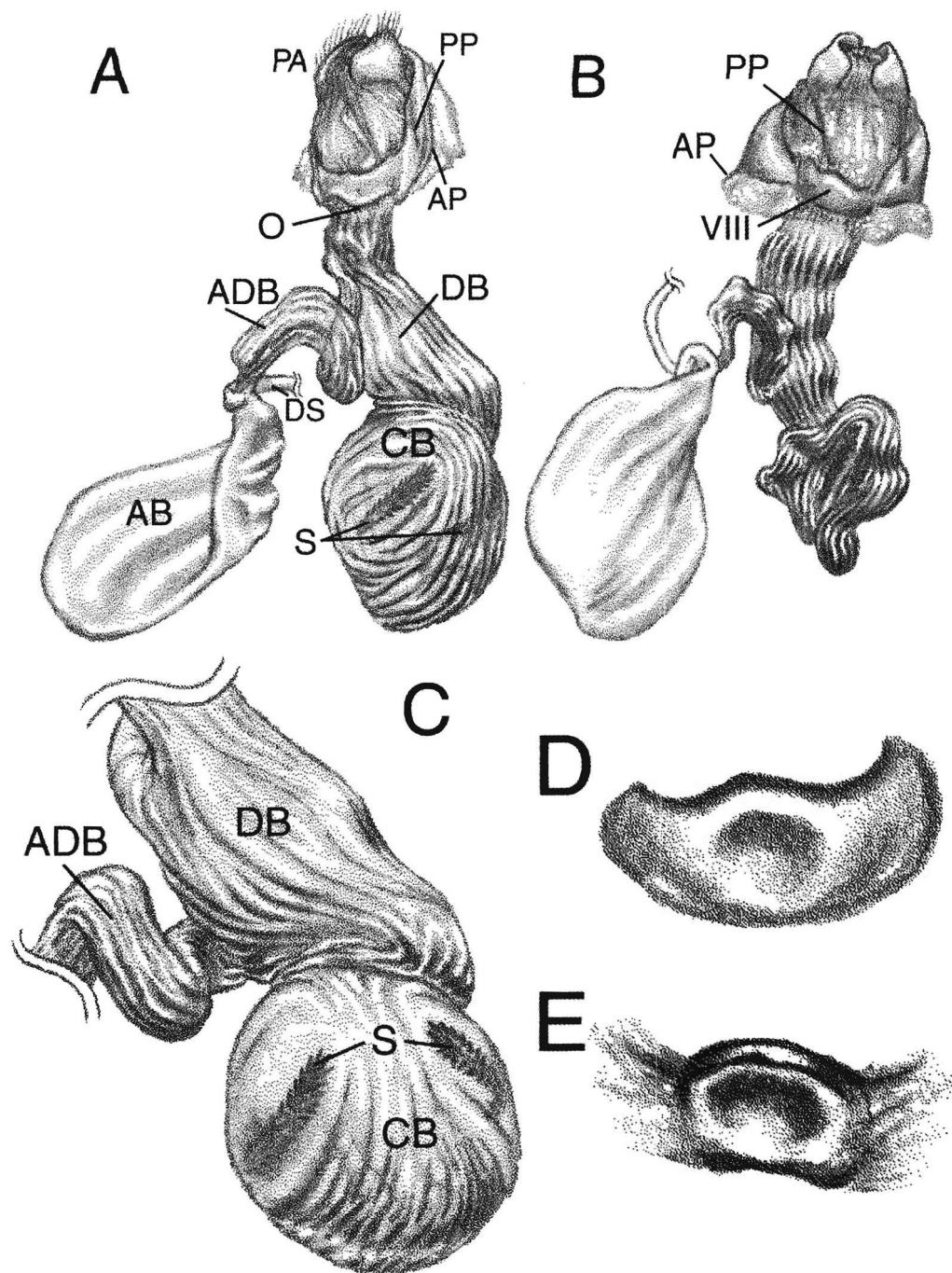


FIG. 4. Female genitalia of: *E. pugione* Puerto Rico culture, SJW1054 (A), *E. pugione* Puerto Rico culture SJW1068 (B), ductus and corpus bursae of *E. anassa*, Jamaica, SJW997 (C), details of lamellae postvaginalis (LPV) of *E. pugione* SJW1068 (D) and LPV *E. anassa* Jamaica, SJW997 (E). AB = appendix bursa, ADB = accessory ductus bursa, AP = anterior apophyses, C = corona of cornuti, CB = corpus bursa, DB = ductus bursa, DS = ductus seminalis, O = ostium, PA = papillae anales, PP = posterior apophyses, S = signa, VIII = 8th sternite, V = vesica.

mimetic species (RBS pers. obs.). The bifid uncus of the males (Fig. 2) is diagnostic for *Empyreuma*, and appears to be unique within the Euchromiini-Ctenuchini clade (sensu Jacobson & Weller 2002). The paired,

spinose signae of the females (Fig. 4S) are also distinctive and possibly derived only in this genus.

Adult habitus (Fig. 1A–C). Wings opaque with brown, black or blue-black scales on upper surface of forewing. Relatively large

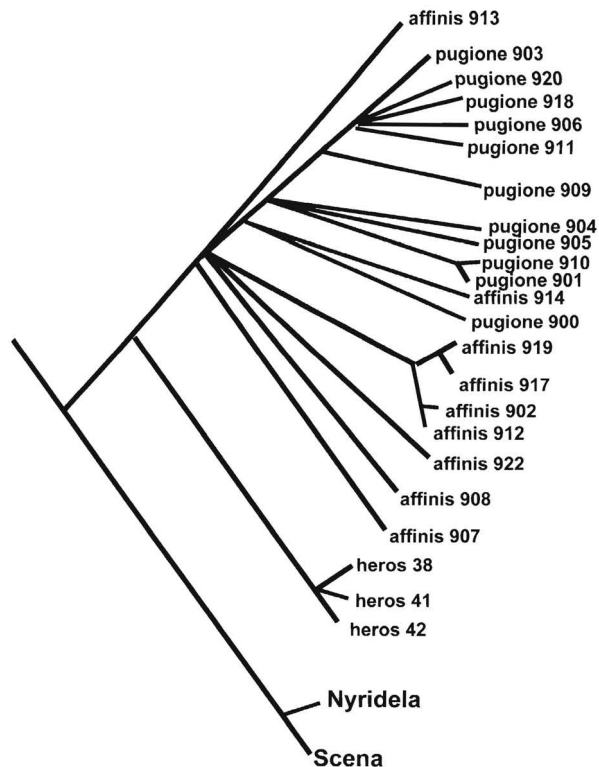


FIG. 5. Maximum likelihood tree with *Neridela* and *Scena* as outgroups. Taxon name includes specimen DNA voucher number (see Table 1).

moths, average male wingspan 49 mm and average wing length 21.8 mm ($n = 82$). In females, average wingspan 50 mm and average wing length 20.5 mm ($n = 90$). Unipectinate antenna with a black shaft for nearly entire length contrasting with orange apex. Ground color of head, thorax, and abdomen black, blue-black, or brownish depending on specimen.

Head and Thorax. Antennae unipectinate; ocelli present with a melanized outer ring. Proboscis longer than head. Prothorax lacks parapatagia or a dorsal gland; ephiphysis short. Both meso- and metathoracic legs possess simple claws (not bifid), and tibial spurs with smooth (not serrated) apices.

Wings (Fig. 6). **Forewing venation:** Sc slightly sinuous, extending nearly 9/10 of costa. R_1 and R_2 arise from discal cell, and R_{3-5} stalked with R_3 arising closer to cell than R_3-R_4 branching. M_1 arises from cell and separate from R_{3-5} . The cross vein between M_1 and M_2 typically thins or even has a break anterior to M_2 . M_2 arises near M_3 with a short spur of M_2 extending into cell. CuA_1 and CuA_2 widely separated and extend to wing edge. **Hindwing venation:** Sc + R_1 absent. RS and M_1 connate. Discal cell cross-vein strongly developed and V-shaped. M_2 weakly developed, and extends to wing margin. M_3 strongly developed, arises from apex of discal cell. CuA_1 and CuA_2 stalked, and Anal vein (A) just touching wing margin. Female with two frenular bristles.

Abdomen. In both sexes, second sternite with long, straight apodemes (Fig. 7), and 2nd and 3rd sternites and tergites lack modifications for wasp mimicry (Weller et al. 2000). Male lacking androconia (no abdominal scent pouch or coremata). 8th sternite weakly sclerotized (Fig. 7).

Genitalia. Males (Figs. 2, 3): **Tegumen** relatively short, arising nearly perpendicular to the vinculum; uncus bifid, long processes

with rounded apices (Fig. 2A–C), or bifid processes short with pointed apices (Fig. 2D); narrow, sinuous vinculum ventrally produced as rounded shallow saccus. **Valve** with strongly sclerotized base and costa; attachment point of M_5 (valve extensor muscle; Forbes 1939, Tikhomirov 1979) marks edge of costa and sacculus (Fig. 2, M_5a); sacculus extending apically as a membranous lobe (distinct from costa) bearing a large, spinose pad on internal surface (Fig. 2A–C, SP; *E. pugione*-complex), or spinose pad reduced to flattened, irregular, rugose area fused to costa (Fig. 2D, SP; *E. anassa*); membranous ventral edge of sacculus with few setae and sclerotized base coincident but not fused to juxta. **Juxta** protruding posteriorly in a bell-shaped projection with wishbone-shaped thickening of edges (Fig. 2A–D); **Anellar region** with small anellar sclerites fused to venter of aedeagus, pair of slender, crescent-shaped patches lie dorsad of aedeagus (Fig. 2, T); anal tube (Fig. 2A) with pair of irregularly shaped sclerites or anal tube lightly sclerotized. **Aedeagus** (Fig. 3) relatively large, compared to genital capsule; endophallus with a sclerotized tube possessing a flattened apex ringed with short spines (Fig. 3); varying number of teeth-like spines, and number not corresponding with species-limits.

Females (Fig. 4A–D): **Papillae anales** (PA) lightly sclerotized, laterally flattened; membrane surrounding ovipore highly folded with melanized striations; posterior apophyses (PP) long and narrow; dorsal pheromone glands as paired narrow tubes with rounded or crescent-shaped thickenings at midpoint and terminus—as long or slightly longer than posterior apophyses (not shown). **8th tergum and sternum** fuse at right angles with very short anterior apophyses (Fig. 4B, AP); 8th tergum rounded, broad with anterior edge highly concave; 8th sternum weakly sclerotized and lacking ornamentation (Fig. 4A–B, detail 4D; *E. pugione* complex), or with a distinct, rounded lamella postvaginalis in *E. anassa* (detail, Fig. 4E); lamella antevaginalis absent; ostium bursa marked by a membranous ventral edge of ductus bursa. **Ductus bursae** (DB) short, membranous and same width as ostium bursa. **Corpus bursae** (CB) with two oblong, highly spinose signae located opposite one another ventrally and dorsally, and linked to an accessory bursa by a twisting duct. **Ductus seminalis** (DS) arising from accessory ductus bursa (ADB). **Accessory bursa** (AB) comprised of thinner membrane, lacking ornamentation.

Notes on Biology. Larvae of these species have been reared on *Nerium oleander* (Apocynaceae) based on museum labels. The plant genus *Nerium* has only three species, and is native from the Mediterranean to Japan. The exotic species, *N. oleander* has been naturalized widely in North America (Correll & Johnston 1979) and it contains cardiac glycosides. The native hosts of *Empyreuma* have not been recorded, but potential New World apocynaceous host genera include *Thevetia* L., *Plumeria* L., *Mandevilla* Lindl., and *Tabernaemontana* L..

Empyreuma anassa has been collected from nearly sea level to 918 m elevation. Similarly, *E. pugione* has been collected up to 733 m. Adults have been collected in April, May, July, September, and December. Historical label data for *E. heros* lacks elevation, but flight times are similar occurring in October, December, and March.

Discussion. *Empyreuma anassa* is easily separated from the *E. pugione* complex, based on color and genital differences. Our examination of the male genitalia suggests that intraspecific variation exists in the shape of the uncus, presence and development of a spinose

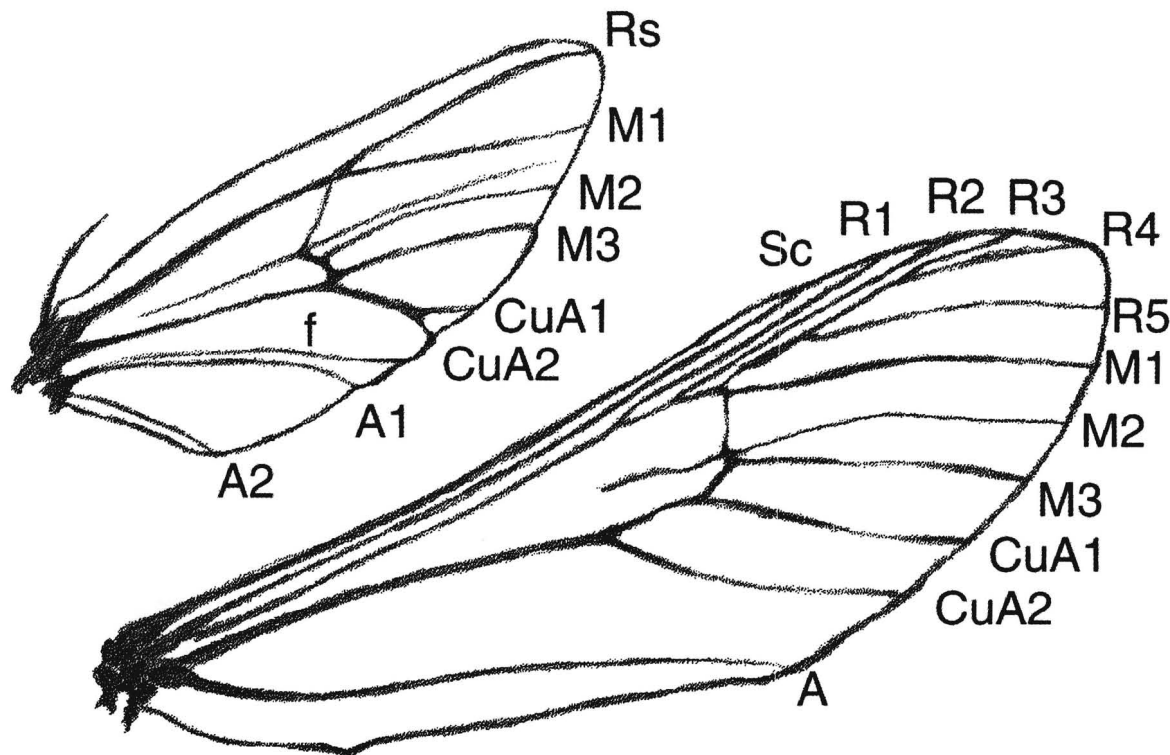


FIG. 6. Wing venation of forewing (below) and hindwing (above). A = anal vein, CuA₁ = cubital vein, f = fold, R = radial vein, Rs = radial sector, Sc = subcosta, 1–5 = vein number.

patch on the sacculus, and presence of a small tooth on the vesica for specimens identified as either *E. pugione* or *E. affinis* (variation not shown). Specimens of the subspecies described by Rothschild (*E. a. haitensis*, *E. a. portoricensis*) fall within the range of morphological variation observed in the reared cultures from Florida and Puerto Rico. Both are placed as new junior synonyms of *E. pugione*. These subspecies may not be defensible; however, more complete survey of moths, their larvae, and their DNA across the Greater Antilles is needed before subspecific status can be discarded definitively, work that is in progress (J. Rawlins in prep).

Empyreuma pugione (Linnaeus, 1767)

Empyreuma pugione (Linnaeus, 1767).

Sphinx pugione Linnaeus, 1767. *Syst. Nat.* (Ed. 12) 1(2):807. Type

Locality: insula S. Thomae [St. Thomas Island, Virgin Islands]

Sphinx lichas Cramer, 1775. *Papillons Exot.* 1:70; pl. 45, fig. B. Type Locality: St. Thomas, Virgin Islands [not *Zygaena lichas* Fabricius, 1781, from Arabia, in the Zygaenidae].

Sphinx sanguinosa Martyn, 1797. *Psyche*: pl. 26, figs. 18, 19 (unavailable?).

Chrysaor erythropterus Hübner, 1808. *Erste Zutrage Samml. Exot. Schmett.*: 4. Unavailable: sole species included in *Chrysaor*, in a work rejected for nomenclatural purposes.

Empyreuma lichas: Hampson, 1898, not Fabricius, 1781. *Cat. Lep. Phal. Brit. Mus.* 1:423; fig. 223. (Misidentification).

Empyreuma mucro Zerny, 1912 (25 July). In Wagner, ed., *Lepid. Catalogus* 7:122 [unnecessary replacement name for *Zygaena lichas* sensu Hampson, 1898, not Fabricius, 1781].

Empyreuma sanguinea Rothschild, 1912 (21 Dec.). *Novit. Zool.* 19:155. [unnecessary replacement name for *Zygaena lichas* sensu Hampson, 1898, not Fabricius, 1781].

Hampson, 1914. *Cat. Lep. Phal. Brit. Mus. Supp.* 1:267 [as valid name for taxon Hampson, 1898:423 had misidentified as *E. lichas*].

Forbes, 1917. *Bull. Amer. Mus. Nat. Hist.* 37:344 [as synonym of *E. pugione*].

Empyreuma sanguinea portoricensis Rothschild, 1912 (21 Dec.). *Novit. Zool.* 19:155. Type Locality: Puerto Rico.

Hampson, 1914. *Cat. Lep. Phal. Brit. Mus. Supp.* 1:267 [as synonym of *E. sanguinea*].

Empyreuma affinis Rothschild, 1912. *Novit. Zool.* 19:155. Type Locality: Cuba; **new synonym**.

Forbes, 1917. *Bull. Amer. Mus. Nat. Hist.* 37:344.

Hampson, 1914. *Cat. Lep. Phal. Brit. Mus. Supp.* 1:267; pl. 13, fig. 31.

Empyreuma affinis affinis Rothschild, 1912. *Novit. Zool.* 19:155. Type Locality: Cuba; **new synonym**.

Empyreuma affinis haitensis Rothschild, 1912. *Novit. Zool.* 19:156. Type Locality: Haiti; **revised synonym** [of *E. pugione* (L.)].

Forbes, 1917. *Bull. Amer. Mus. Nat. Hist.* 37:344 [as valid "race" of *E. affinis*; misspelled as "haytiensis"].

Hampson, 1914. *Cat. Lep. Phal. Brit. Mus. Supp.* 1:267 [as synonym of *E. affinis*].

Empyreuma haytiensis Forbes, 1917. *Bull. Amer. Mus. Nat. Hist.* 37:339, 344. Misspelling.

Diagnosis. The wing length in males ranges from 19–27 mm (A = 21.8; STD = 2.5; n = 24), and is 17–27 mm in females (A = 19.9; STD = 2.4; n = 35). The forewing varies from primarily brownish red with

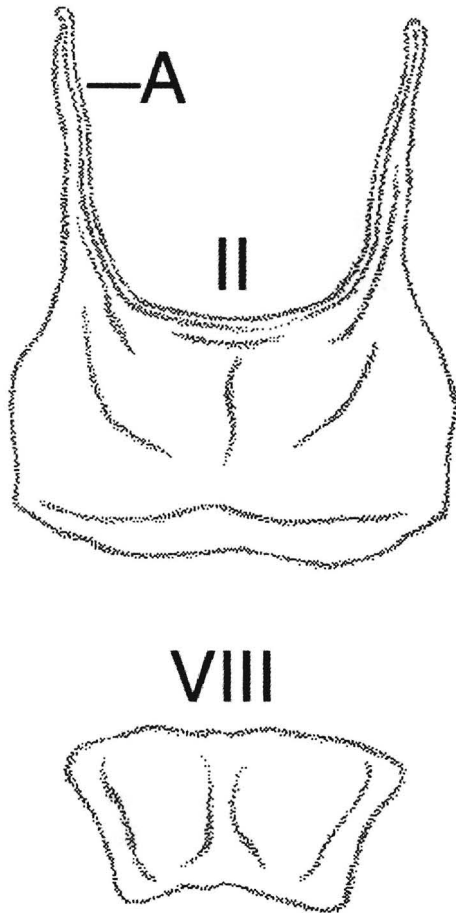


FIG. 7. Male abdominal sternites of *E. pugione* (SJW1005): second (II) above, eighth (VIII) below.

costal red streak (Dominican Republic, Haiti) to brownish red with orange costal streak (Cuba), and has a thin black border on the outer margin (Fig. 1A, B). The forewing lacks scarlet coloration on the underside. The black border varies in width between individuals. White markings are present on the thorax, the patagia, the tegulae, and the wing bases. These white markings are more strongly developed in specimens from Bahamas. Medial, dorsal white patches are present on A1 and A2. There is a small pair of white spots on A2 in pleural region. Conspicuous, paired white patches are present on the dorsum of A3 and A4. Smaller than those occurring on A3 and A4, white patches occur on the anterior edge of A5–A7. Pleural spots are present on A3–5 and A7–8. These white patches are missing or reduced in some specimens and patch size varies throughout the range. The tegumen is relatively short, arising nearly perpendicular to the vinculum. The uncus is bifid, possessing long processes with rounded apices (Fig. 2A–C). The sac-

culus extends apically as a membranous lobe (distinct from costa), and bears a large, spinose pad on internal surface (Fig. 2A–C, SP). The anal tube has a pair of irregularly shaped sclerites (Fig. 2A). The 8th sternum is weakly sclerotized, and lacks ornamentation (Fig. 4A–B, detail 4D).

Specimens examined: (see Table 1 for additional genitalic dissections). CUBA: **Baracoa**, Coll. Wm Schaus, March (5♂; NMNH). **Cayanas**, E.A. Schwarz (1♂; NMNH). **Holguin**, no other data (1♂, 2♀; BMNH); BM2663 (1♂; BMNH); ex Ges Franek 1911 (1♀; BMNH); H.S. Parish (1♀; BMNH). **Hayana**, Baker (2♂; NMNH). **Loma del Gato**, Sierra Maestra Cuba, 2500', July–August [19]29, HF Clement (2♂, 1♀; BMNH); same locality: BM2465 (1♂, BMNH). **Santiago**, F. Clement (1♂; NMNH). Parish *affinis* syntypes (2♀; BMNH); 58-126 (1♀; BMNH); 100-20 (1♂, 1♀; BMNH); 61-21 (1♂; BMNH); *affinis* syntype 2455 (1♂, BMNH). BRITISH VIRGIN ISLANDS: **Guiana Island**, 1–14 July 1984, SE & PM Miller (1♂; gen. prep. SJW1004; FSMC). **Tortola**, March [19]66 J.A.C. Greenwood (1♀; BMNH). DOMICAN REPUBLIC: **La Vega Prov.**, Hotel Montana, ca. 520 m, 10 km NE Jarabacoa, 28 May 1973, Don & Mignon Davis (1♂; gen prep SJW1005, NMNH). CAYMAN ISLANDS: **Georgetown**, T.M. Savage English 1911-89 (1♂; BMNH); no other data (1♀, BMNH). GUADELOUPE: **Aer. Du Raizet**, ix 81 R. Gisointeties (1♀; BMNH). HAITI: **Petionville**, 1300' 26 Dec. 1954 A. Zerkowitz (1♀, BMNH). **Port au Prince**, 24 X [19]09 Georges Lion, Museum Paris (2♂; MNHP); same locality, 26 X [19]09 Georges Lion, Museum Paris (1♂; MNHP); same locality, 28 X [19]09 Georges Lion, Museum Paris (1♂; MNHP); F. Odile Joseph (1♂, 1♀, 1♂; gen prep 2462; BMNH); No. 54-28, Nov. 26, 1928, A. Audant coll. (1♂; gen prep SJW1003; NMNH). **Le Perchoir**, 3000' 21 Dec. 1954 A. Zerkowitz (1♀; BMNH). same locality, 23 Dec. 1954 A. Zerkowitz (1♂; BMNH); same locality, 26 Dec. 1954 A. Zerkowitz (2♂, 3♀; BMNH); G. Babault (1♀; BMNH). **San Domingo**, syntype F *affinis haitiensis* (1♀; BMNH); same locality, syntype ♂ *affinis haitiensis* (1♂; BMNH); Haiti, syntype ♂ (BMNH); Haiti 98, BM2459, syntype ♀ (BMNH). HONDURAS: (1♂; BMNH). Haiti (2♂; BMNH). ST. DOMINGO: Tweede 55-1, BM2426 (1♂, BMNH). **NO DATA**. Ex Musaeo Arch. Guenee (1♂; MNHP); Brazil Ex. Coll. Smith 1894-5 (1♀; BMNH); America (1♀; BMNH); Savanah *Zyganea lichas* (1♀; BMNH); *Zyganea pugione* Haynes S.P. Oss-Terra (1♀; BMNH). No label (2♂, 1♀; BMNH). *Empyreuma pugione* L. (1♀, BMNH); *pugione* L. (1♀; BMNH); (2♂; NMNH); "Type of *sanguinea* from S. Domingo?" (1♂; BMNH); Coll. Bryk Mus. (3♂; NMNH); orn 2 on 11/69 (1♂; BMNH).

Empyreuma heros Bates, 1934

Empyreuma heros Bates, 1934. Occ. Pap. Boston Soc. Nat. Hist. 8:137. Type Locality: Bahamas (Mariguana Island).

Diagnosis. This species can be diagnosed based on its collection locality and its COI sequence. Unique substitutions are given in Table 2.

Description. Same as *E. pugione*.

Specimens examined. BAHAMAS: **Crooked I**, vic Pitts Town, 25 ix 1986 M. Simon & L. Miller, Sta. 1986–UV (1♂; gen prep SJW1001, 1♀; gen prep SJW1083; FSMC); 1 mi E. Colonel Hill, UV, 18 ix 1988, LD Miller & MJ Simon, Sta. 1988-45, Acc. 1988-18, (1♂; gen prep SJW1082; FSMC). **Grand Turk Is[land]**, Xii-1. 1965–66 (1♀; BMNH). **G[reat] Inagua Is[land]**, Horse Pond ca 1.5 km E of Matthew Town, 28 ix 1986, M. Simon & L. Miller, Sta. 1986-19 (1♂; gen prep, SJW1002; FSMC). **L. Abaro**, Mar 1902 (1♀; BMNH). **Long Island**, Stella Maris, UV, 26 ix 1988, L.D. Miller & M.J. Simon Sta. 1988-63 (2♂; gen prep SJW1000, SJW1081; FSMC). **Nassau Is[land]**, 19 ii [19]02, J.L. Bohnhote (2♂, 2♀; BMNH); J.L.

Bohnnote (1♀; BMNH); I. 22 X [18]98 (1♀; BMNH); G. Carter 1903-6 (1♀; BMNH); 30 Dec. 1956, A. Zerkowitz (1♂; BMNH). **New Providence Is.**, F.E. Taylor (1♂, 1♀; BMNH).

Empyreuma anassa Forbes, 1917

Empyreuma anassa Forbes, 1917. Bull. Amer. Mus. Nat. Hist. 37:343; fig. 5. Type Locality: near Troy, Jamaica.

Empyreuma pugione: Hampson, 1898 (not Linnaeus, 1767). Cat. Lepid. Phal. Brit. Mus. 1:423. Misidentification.

Diagnosis. *E. anassa* appears slightly larger than *E. pugione*, because the body is more robust. The male wing length ranges from 16–26 mm ($A = 21.1$; $STD = 1.9$; $n = 56$); female wing length ranges from 16–25 mm ($A = 21$; $STD = 2.0$; $n = 44$). Like *E. pugione*, the upperside of the forewing is opaque with metallic blue-black or brown scales. In *E. anassa*, however, the underside is scarlet with a large black border on the outer margin, and the scarlet coloration is visible dorsally (Fig. 1C). Unlike *E. pugione*, white scales are lacking on the thorax and legs in *E. anassa*. The abdomen has two white crescent bands on A4 that extend to the spiracles but do not meet dorsally. There are smaller, paired bands on A5–A7. Male genitalia also differ between the two species. The bifid uncus of *E. anassa* is short with pointed apices (Fig. 2D) compared to *E. pugione* (Fig. 2A–C). The spinose pad on the sacculus is reduced to a flattened, irregular, rugose area, which is fused to the costa (Fig. 2D, SP). The anal tube is lightly sclerotized in *E. anassa*, but not *E. pugione*. In females, the 8th sternite has a distinct, rounded lamella postvaginalis (Fig. 4E) compared to the trapezoidal one of *E. pugione* (Fig. 4D).

Species distribution. The species is found in Jamaica. Three specimens had erroneous label data: British Guiana, Cuba and Costa Rica. The “Moneague” locality is a famous collecting locality in St. Ann Parish Jamaica (Brown & Heineman, 1972), not Costa Rica. The other two specimens lack additional information (collector or expedition) that would allow definitive assignment of locality. These are treated as mislabeled because no other specimens of *E. anassa* have been taken outside of Jamaica.

Specimens examined (also Table 1). BRITISH GUIANA: **Paruima**, 26. 10. 38 (1♀; BMNH). COSTA RICA [Jamaica St. Ann]: **Moneague**, I iv 1926 1000 ft. F. W. Jackson (1♀; BMNH). CUBA: **Holguin**, (1♂; BMNH). JAMAICA: **Axe Town**, Bred 7.8.[18]92 (Taylor) (1♀; BMNH). **Bath**, USNM Acc 40269 (1♀; NMNH). **Jamaica: Batton Falmouth**, AGM Ja.–Feb. 1967 (1♂; BMNH). **Clar. Par.**, Portland Ridge, nr Jackson Bay Cave, 40 ft 4 May 1973, Don & Mignon Davis (5♂, 1♂; gen prep SJW999; NMNH); Mason River Station, 4 mi NW Kellits, 2200 ft 16–19 April '73, Don & Mignon Davis (1♂, 1♂; gen prep SJW1084; NMNH); 2 Km S. Rocky Pt, nr Jackson Bay Cave, Dec 10, 1975 5m, Don & Mignon Davis (2♂; NMNH). **Constant Springs**, e. xii 1904 Wlsm. (1♀; BMNH). **Cuna Cuna Pass**, Capt. U. Robinson Collector 10 July [19]05 (1M; NMNH). **Cornwall County**, Coll. Miss M.S. Savarian (1♀; NMNH). **Kingston**, 10-1-[18]94 at light (Taylor) (1♀; BMNH); at

light (Taylor) (1♀; BMNH); bred 2.8.[18]92 (Taylor) (1♂; BMNH); bred 6.8.[18]92 (Taylor) (1♂, 1♀; BMNH); bred 7.8.[18]92 (Taylor) (1♂; BMNH); bred pupated 7.7.[18]92 emerged 20.7.[18]92 no. 10 *E. pugione* (Taylor) (1♀; BMNH); WJ Kaye B. M. 1930-185 (1♂; BMNH); at light 29-7-[18]92 (1♂; BMNH); 15-IV-73 (1♂; NMNH); W.R. Maxson Coll. V-29-04 (1♂; NMNH). **Mandeville**, 2000 m July 1923 A. Hall (1♂; BMNH); L. J. Bertram (1♂; BMNH). **Mile Gully**, 16 ii 1921 No. 311 (1♀; BMNH). **Moneague**, beg. Feb. 1905 Wlsm. (1♀; BMNH). **Montego Bay**, xiii 1923 Major Gillett BM 1924-174 (2♀; BMNH); 26 Feb. 1911 Miss Fountaine (2♂, 1♀; BMNH). **Moore Town**, (1♂; BMNH). **North Coast**, s. level 20 iii 1961 (1♀; BMNH); s. level 29 iii 1961 (1♂; BMNH); s. level 8 vi 1961 (2♂, 1♀; BMNH); Dark hours s. level 20 iii 1961 (2♂; BMNH); Dark hours s. level 29 iii 1961 (1♂; BMNH). **Ocho Rios**, Dec. 10, 1957 A. Zerkowitz (1♂; BMNH); Dec. 28, 1957 A. Zerkowitz (1♀; BMNH); Jan. 3, 1957 A. Zerkowitz (1♂, 1♀; BMNH); Jan. 5, 1957 A. Zerkowitz (1♂; BMNH); Jan. 6, 1957 A. Zerkowitz (1♂, 1; BMNH). **Portland Parish**, (1♂; BMNH); Hardwar Gap, “Green Hills”, July 24–25, 1962, Farr. O & R. Flint (5♂, 1♀; NMNH); 4 mi. S. Hartford, 850 ft. 26–27 April 1973, Don & Mignon Davis (1♀; NMNH). **Runaway Bay**, end Feb. 1905 Wlsm (1♀; BMNH); 5-III-1970, JFG Clarke (1♀; NMNH). **St. Ann Par.**, Martin (1♂; BMNH); Rose Hill, Runaway Bay 900 ft, 29 April–2 May 1973, Don & Mignon Davis (13♂, 4♀; NMNH); nr Runaway Bay 50 ft, 1–2 May 1973, Don & Mignon Davis (6♂, 1♀; NMNH). **St. Cath. Par.**, Mt. Diablo Hollymount, 2754 ft 21–24 April '73, Don & Mignon Davis (5♂, 1♀, 1♂; gen prep SJW998; NMNH). **Westmor. Par.**, Negril, Dec. 12, 1975, Don & Mignon Davis (2♂; NMNH). BRITISH WEST INDIES: **N Coast**, S level 20 iii 1961 (2♂, 1♀; BMNH); same locality: 29 iii 1961 (1♂, 1♀; BMNH); same locality: 8 vi 1961 (2♂, 1♀, 1♂; gen prep SJW996; BMNH). ST. THOMAS: **L. Litus**, *pugione* (1♀; BMNH); Jamaica collection Wm Schaus (5♂, 3♀; NMNH); Female vial #083 *Empyreuma anassa* R.E. Dietz 1968 (1♀; NMNH); Jamaica WI, A. Arinoff Donor, July 24, 1933 (1♂; NMNH); Cockrell (1♀; NMNH); St. Thomas Col. Neumögen, Coll. Brklyn Mus. (1♂; NMNH); *Empyreuma pugione* 4. 8. 25 Ex. Coll. Griffiths (1♀; BMNH); *Empyreuma pugione* Jamarque (1♂; BMNH); (6♂, 8♀; BMNH); Taylor (6♂, 4♀; BMNH); Jamaica Yates (1♂; BMNH); 1960 pres. by George Newman Brit. Mus. 1961-52 (1♀; BMNH); 40-4-3-104 (1♂; BMNH); 45-110 (1♂, 1♀; BMNH); 46-121 (1♂; BMNH); 76-71 (1♂; BMNH); E Coll. Hanson (1♂; BMNH); ex Stevens (1♀; BMNH); F. W. Jackson 1913-208 (1♂; BMNH); F. W. Jackson 1920-341 (1♂; BMNH); Ianson (1♀; BMNH); JMS and J Yates BM 1926-393 (1♂; BMNH); ex Percy I. Lathy 1902 (1♂; BMNH); (1♂; BMNH); P.H. 6-91 Taylor (2♂, 1♀; BMNH); R. Stanway Paris 23-ii-1919 (1♀; BMNH); same data 2-ii-1919 (2♂, 1♀; BMNH); same data 16-2-1901 (1♀; BMNH); West Indies Malrun B. M. 1933-489 (1♂, 1♀; BMNH); Malvun (1♂; BMNH); British Guiana (1♂; BMNH); San Domingo 120 (1♂; BMNH). NO DATA: Bred 13. 7. [18]92 No. 10 *E. pugione* (1♂; BMNH); Bred 25. 7. [18]92 No. 10 *E. pugione* (1♂, 1♀; BMNH); 44-11 (1♂; BMNH); *E. pugione* no. 10 Bred 16. 7. [18]92 (1♀; BMNH); Panama or Jamaica ? (1♀; BMNH) S. America (1♀; BMNH); label unreadable (1♂; BMNH).

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