

A NEW SPECIES OF *ACTINOTE* HÜBNER FROM THE EASTERN ANDES
OF ECUADOR (LEPIDOPTERA: NYMPHALIDAE: HELICONIINAE)

KEITH R. WILLMOTT, ANDRÉ V. L. FREITAS, JASON P. W. HALL,
KARINA L. SILVA-BRANDÃO, AND MARLON PALUCH

(KRW) McGuire Center for Lepidoptera and Biodiversity, Florida Museum of Natural History, University of Florida, Gainesville, FL 32611, U.S.A. (e-mail: kwillmott@flmnh.ufl.edu); (AVLF) Departamento de Zoologia, Instituto de Biologia, Universidade Estadual de Campinas, CP 6109, Cep 13083-970, Campinas, SP, Brazil; (JPWH) Department of Entomology, National Museum of Natural History, Smithsonian Institution, Washington, DC 20560-0127, U.S.A.; (KLS-B) Departamento de Entomologia, Fitopatologia e Zoologia Agrícola Esalq-USP, Av. Pádua Dias 11, Caixa Postal 9, Cep 13418-900, Piracicaba, SP, Brazil; (MP) Departamento de Zoologia, Universidade Federal de Pernambuco, Recife, PE, Brazil

Abstract.—A new species of *Actinote* Hübner, *A. kennethi* Freitas, Willmott and Hall (Lepidoptera: Nymphalidae: Heliconiinae), is described from cloud forest habitats in eastern Ecuador. Molecular sequence data and morphological characters both indicate that the new taxon is closely related to *Actinote g. genitrix* d'Almeida, 1922, from southeastern Brazil, and the Venezuelan *Actinote genitrix costae* Neild, 2008. Based on the substantial geographic isolation of these taxa, observed morphological differences and mtDNA sequence divergence, we argue for the treatment of the new Ecuadorian *Actinote* taxon as a distinct species.

Key Words: *Actinote*, Brazil, Ecuador, morphology, mtDNA sequence data

The tribe Acraeini is a compact group within the Heliconiinae, a nymphalid subfamily characterized by distinctive morphology in both the adult and immature stages (Ehrlich 1958, Penz and Peggie 2003, Freitas and Brown 2004). The tribe contains approximately 220 species distributed throughout the Afrotropical and Neotropical regions, with a few species also occurring in southeastern Asia and Oceania (Pierre 1987, Lamas 2004). The genus *Actinote*, *sensu stricto*, as defined by Paluch (2006), constitutes a clade within the otherwise African and Asian genus *Acraea* Fabricius, 1807 (Penz and Peggie

2003, Silva-Brandão *et al.* 2008b) and is represented by 31 exclusively Neotropical species (Francini *et al.* 2004, Lamas 2004, Paluch 2006, Paluch *et al.* 2006). All species for which the immature stages are known have gregarious larvae and feed exclusively on plants of the family Asteraceae (Francini 1989, Francini *et al.* 2005).

According to current knowledge, the peak of *Actinote* diversity is in southeastern Brazil, which harbors 21 species, with up to 13 species occurring at a single site (Francini 1989). Adults of *Actinote* often appear exceedingly similar, and females of some species are morphologically practically indistinguishable (Fran-

* Accepted by David R. Smith

cini 1989, Francini and Penz 2006, Paluch 2006). Moreover, most high elevation species have restricted flight seasons and periods and are extremely localized, making them difficult to collect and resulting in poor representation in most collections (Francini 1989, Penz and Francini 1996, Francini *et al.* 2004, Francini *et al.* 2005). As a result of these phenotypic and biological traits, many new species have recently been discovered and/or described from southeastern Brazil (Penz and Francini 1996, Francini *et al.* 2004, Freitas unpublished data) and to a lesser extent Peru (Lamas 2003), Venezuela (Neild 2008) and Bolivia (Paluch *et al.* 2006).

As a result of intensive collecting efforts in the eastern Andes of Ecuador by KRW and JPWH, as part of a long-term inventory of the butterflies of that country (see www.butterfliesofecuador.com), five male specimens of an undetermined *Actinote* taxon have been discovered. A recently collected specimen was sent to AVL and KLSB for molecular analysis and inclusion in a molecular phylogeny of the genus (Silva-Brandão *et al.* 2008b). In this analysis the Ecuadorian *Actinote* proved to be most closely related to *Actinote genitrix* d'Almeida, 1922, a species then known only from southeastern Brazil (but see Discussion below). Wing pattern and morphological characters confirmed the relationship inferred from molecular data. In this paper, we describe this new taxon and discuss its systematic position within the genus *Actinote*.

METHODS

The following collections were examined by MP as part of a taxonomic revision of *Actinote*: Instituto Oswaldo Cruz, Rio de Janeiro, Brazil; Museu Nacional, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil; Museu de Zoologia, Universidade de São Paulo, São Paulo, Brazil; Departamento de

Zoologia, Setor de Ciências Biológicas, Universidade Federal do Paraná, Curitiba, Brazil; Museu de Zoologia da Unicamp, Campinas, Brazil; and Museo de Historia Natural, Universidad Nacional Mayor de San Marcos, Lima, Peru. Extant type specimens or their photographs were examined by MP for all names in *Actinote* (*sensu* Lamas (2004) and Paluch (2006)), as listed by Lamas (1996, 2004). The following additional acronyms are used in the text: FLMNH – McGuire Center for Lepidoptera and Biodiversity, Florida Museum of Natural History, University of Florida, Gainesville, Florida, USA; KJWH – Keith R. Willmott and Jason P. W. Hall collection, Gainesville, Florida, USA; and MECN – Museo Ecuatoriano de Ciencias Naturales, Quito, Ecuador.

Genital dissections followed standard techniques, and morphological terms for genitalia follow Klots (1956) and Eliot (1973). Terminology for wing venation follows Comstock and Needham (1918), with cells named for the veins bounding the cell. Light microscopy examination was done using a Leica MZ 12.5 at up to 100 \times magnification.

Molecular analyses used the *Actinote* data set and methodology for extraction, amplification, sequencing and alignment of DNA employed by Silva-Brandão *et al.* (2008b) to propose a phylogenetic hypothesis for the tribe Acraeini. A 648 bp fragment of *COI*, the “barcode” region, was sequenced using the methods of Silva-Brandão *et al.* (2008a) and used here for phylogenetic distance estimation. Neighbor-Joining (NJ) analysis (Saitou and Nei 1987) was applied to obtain graphically the phenetic distance within and between species of *Actinote*. MEGA 3.1 (Kumar *et al.* 2004) was applied for distance matrix computation using the K2P method and for the reconstruction of NJ trees. The robustness of each branch of the NJ trees was determined using the non-parametric

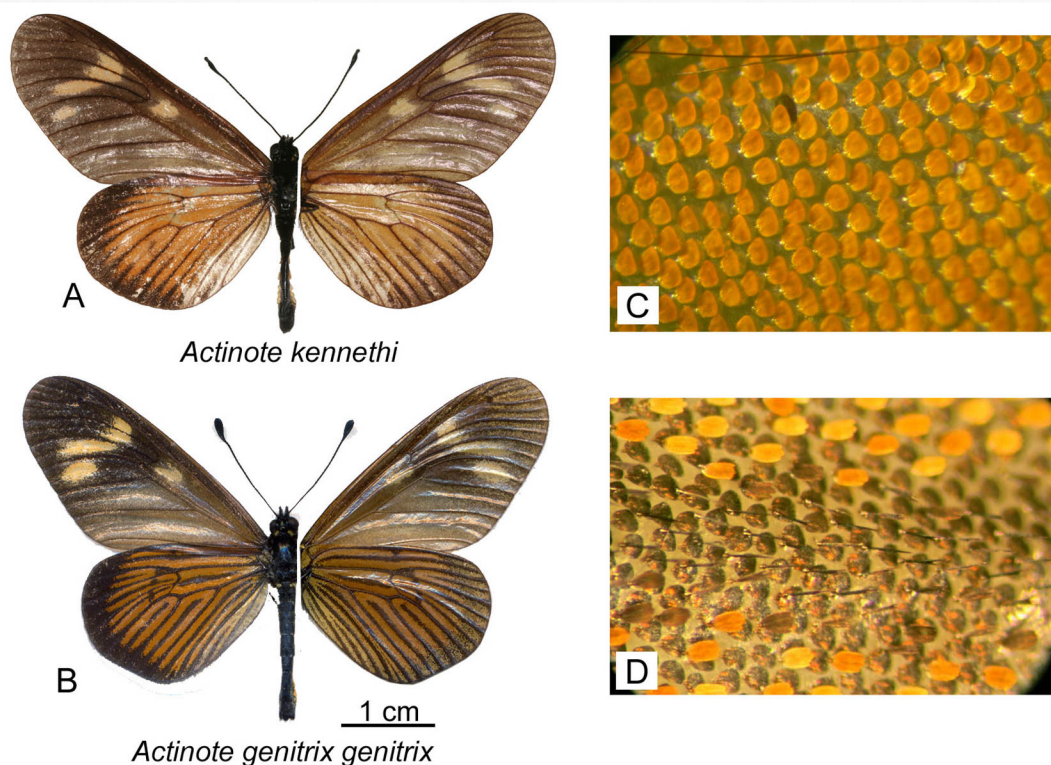


Fig. 1. A, *Actinote kennethi*, holotype male, Río Abanico, Morona-Santiago, E. Ecuador. B, *A. g. genitrix*, male, Alto da Serra, São Paulo, S. E. Brazil. C, *A. kennethi*, scales in the discal cell of the dorsal hind wing. D, *A. kennethi*, needlelike scales within the black intervenal stripe in the apex of the ventral forewing.

bootstrap test (Felsenstein 1985) with 1000 replicates.

***Actinote kennethi* Freitas, Willmott and Hall, new species**
(Figs. 1, 2, 4)

Diagnosis.—*Actinote kennethi* is superficially very similar to a number of other *Actinote* species, especially from southeastern Brazil. However, it shares a number of morphological synapomorphies with the Brazilian *Actinote g. genitrix* and the Venezuelan *A. genitrix costae* Neild, 2008 and *A. romeroi* Neild and Costa, 2008, that assist in its identification. These include the shape of the valva, which is narrow, elongate, tapering, and straight in dorsal view (a unique synapomorphy), the near absence of

scales in and around the discal cell of the ventral hind wing in males (a unique synapomorphy), and the presence of needle-shaped scales in the black intervenal stripes of the ventral forewing apex (a local synapomorphy, also present in *A. canutia* (Hopffer, 1874), *A. mamita* (Burmeister, 1861) and *A. surima* (Schaus, 1902)). The last two characters enable the species to be distinguished without dissection from other north and central Andean *Actinote*, including the recently described Bolivian species *A. mirnae* and *A. mielkei* (Paluch *et al.* 2006). *Actinote g. genitrix*, *A. g. costae* and *A. kennethi* appear to form a further clade, differing from *A. romeroi* in having a narrower subapical FW band that does not continue to the tornus and reduced or absent yellow-orange markings in the basal half of the FW.

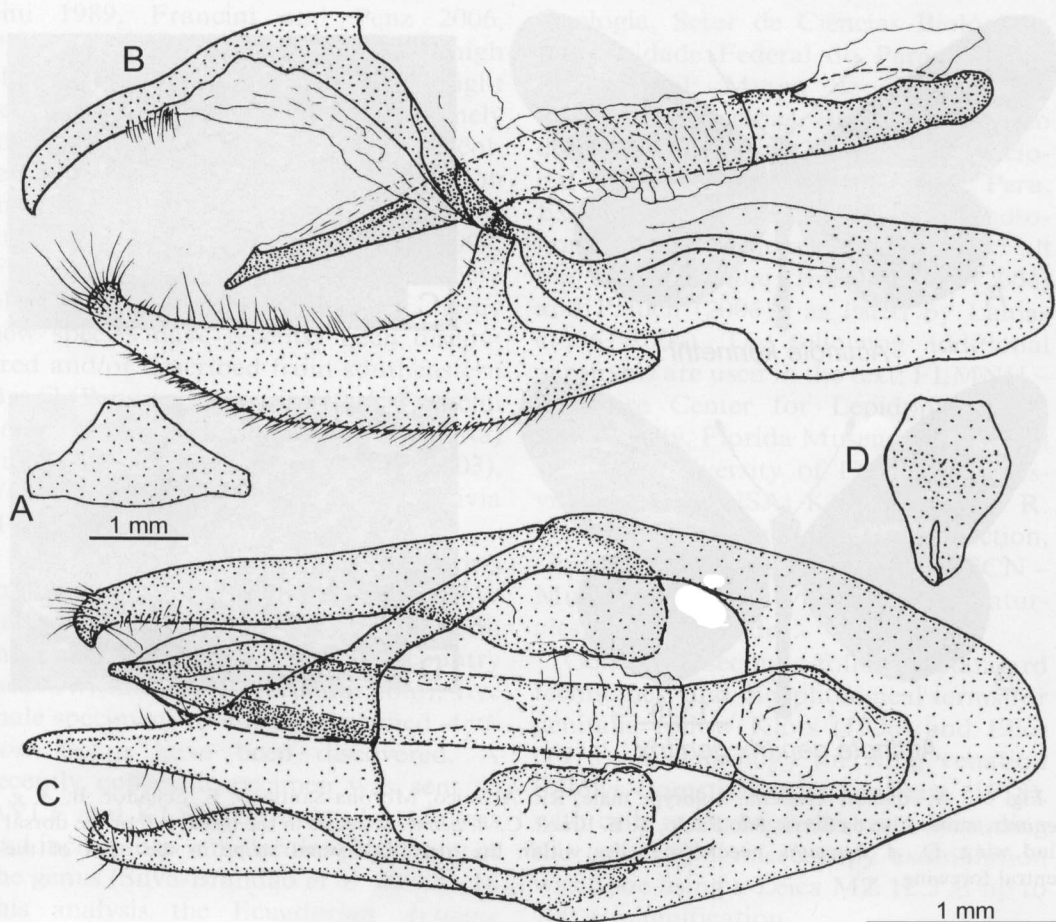


Fig. 2. Male genitalia of *Actinote kennethi* (paratype). A, Eighth sternite in ventral view. B, Genitalia in lateral view. C, Genitalia in dorsal view. D, Juxta in posterior view.

Actinote kennethi differs from *A. g. genitrix* by having a slightly pinkish instead of pale yellow tinge to the forewing subapical band (in fresh specimens), a smaller intradiscal spot on the forewing, slightly more extensive orange markings on the hind wing, and much reduced black intervenal markings on the ventral hind wing. *Actinote genitrix costae* differs from *A. kennethi* by having more extensive, paler yellowish forewing subapical markings, a narrower, crescent-shaped, yellowish intradiscal spot on the forewing, and a broader black margin, paler orange markings, and more extensive black intervenal stripes on the dorsal hind wing.

Description.—Male. Forewing length of holotype 32.7 mm. *Wing shape and venation:* Forewing elongate, rounded at apex, distal margin angled at end of vein M_2 then approximately straight to end of vein $2A$; hind wing apex and tornus smoothly rounded, distal margin slightly bulging between end of veins M_3 and M_2 ; hind wing veins M_1 and R_s originating independently from discal cell, discocellular vein $1d$ present. *Dorsal surface:* Ground color of both wings blackish brown, both wings faintly translucent throughout, with forewing becoming more translucent in basal half of cells $2A-Cu_2$, Cu_2-Cu_1 and discal cell; roughly quadrate, pale salmon-pinkish

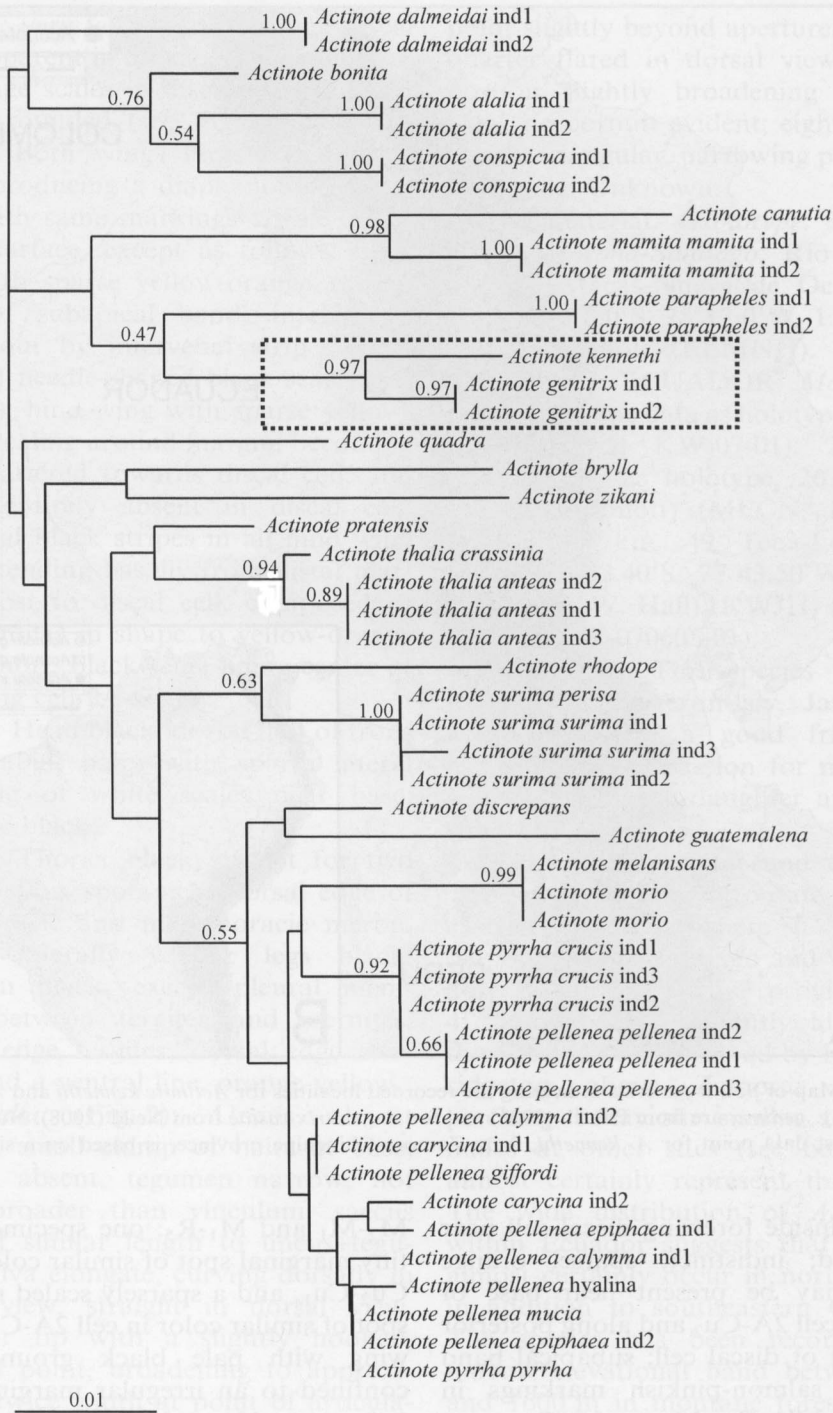


Fig. 3. Neighbor-joining tree constructed with K2P distances of mtDNA *COI* for *Actinote* species and subspecies. The values on the branches indicate bootstrap values.

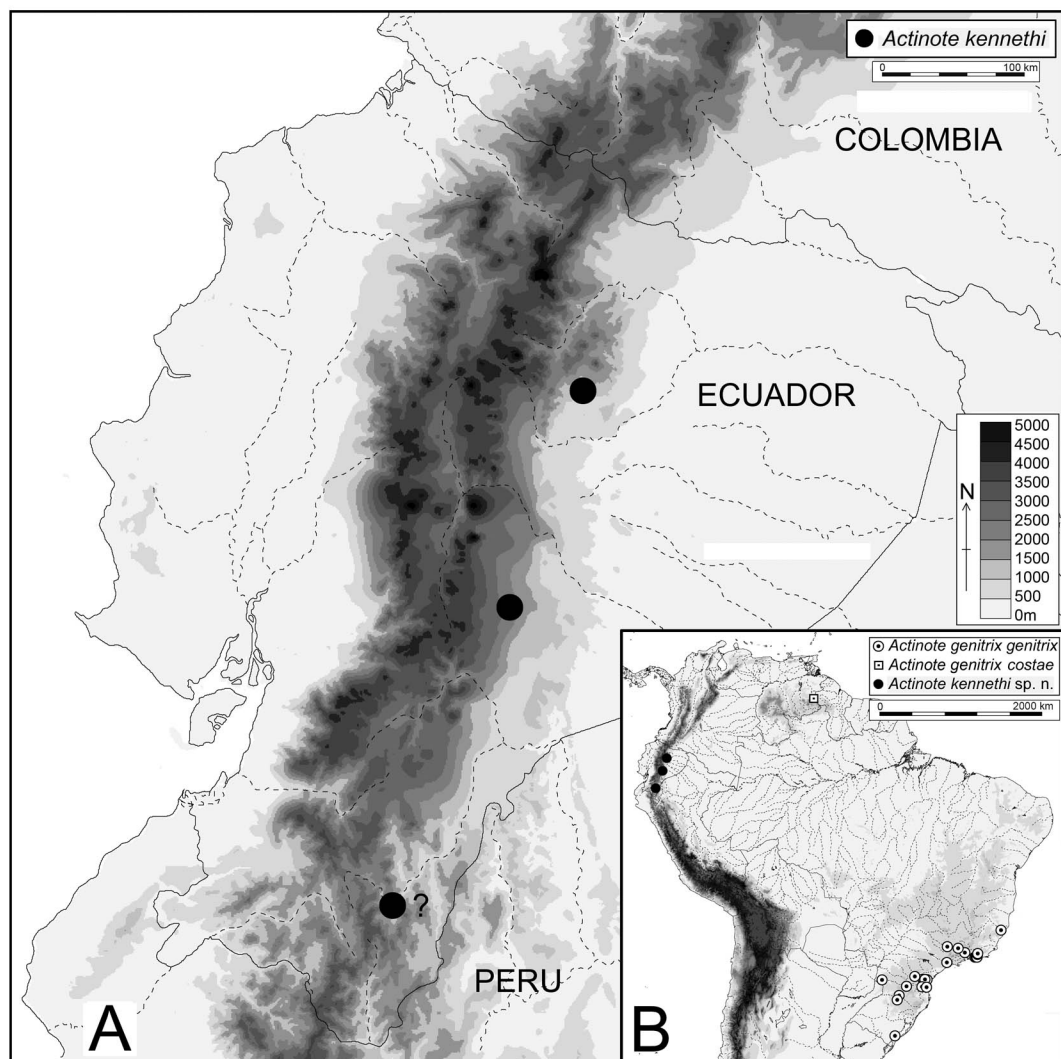


Fig. 4. Map of South America indicating the recorded localities for *Actinote kennethi* and *A. genitrix*. Data for *A. g. genitrix* are from Paluch (2006) and for *A. genitrix costae* from Neild (2008). Note that the southernmost data point for *A. kennethi*, from Zamora-Chinchipe province, is based on a sight record by KRW.

marking inside forewing discal cell near distal end; indistinct, sparse, orange scaling may be present near base of forewing cell 2A-Cu₂ and along posterior basal half of discal cell; subapical band of pale, salmon-pinkish markings in forewing cells Cu₁-M₃ to M₁-R₅ comprising an isolated, small, oval spot in cell Cu₁-M₃, an almost isolated, elongate, rounded dash in cell M₃-M₂, and two contiguous oblong markings in cells

M₂-M₁ and M₁-R₅; one specimen has a tiny marginal spot of similar color in cell Cu₂-Cu₁, and a sparsely scaled marginal spot of similar color in cell 2A-Cu₂. Hind wing with pale black ground color confined to an irregular marginal band 1–3 mm wide, which has prominent intervenal stripes extending basally up to 3 mm; remainder of hind wing dull brownish orange, becoming slightly paler in anal margin, except for veins outlined

in black; very faint trace of black discal band apparent in two specimens; brownish-orange scales in discal cell oval and distally rounded (see Fig. 1C). *Ventral surface*: Both wings largely devoid of scales, producing a diaphanous appearance, with same markings visible as on dorsal surface, except as follows: forewing with sparse yellow-orange scaling distal of subapical band, interrupted throughout by intervenal stripes composed of needle-shaped black scales (see Fig. 1D); hind wing with sparse yellow-orange scaling around margin, becoming more scattered towards discal cell, and almost entirely absent in discal cell; intervenal black stripes in all hind wing cells, extending basally from distal margin almost to discal cell, composed of scales similar in shape to yellow-orange scales; short, black setae lining center of hind wing cell 2A-Cu₂.

Head: Head black; dorsal half of frons white; labial palpi with sparse lateral scattering of white scales near base; antennae black.

Body: Thorax black, except for two lateral yellow spots near dorsal edge of metathoracic and mesothoracic meron, patagia laterally yellow; legs black; abdomen black, except pleural membrane between tergites and sternites, ventral edge tergites, dorsal edge sternites, and a ventral line, orange-yellow.

Genitalia (Fig. 2): Uncus almost straight, small clump of hairs at base; gnathos absent, tegumen narrow, not much broader than vinculum; saccus deep, of similar length to uncus+tegumen; valva elongate, curving dorsally in lateral view, straight in dorsal view, posterior tip with a slightly hooked, rounded point, broadening to approximately twice width at point of articulation with vinculum, sparsely hairy on inner surface and posterior tip; juxta elongate pentagonal, tapering ventrally; aedeagus approximately straight, tapering gradually posteriorly to end in blunt

point slightly beyond aperture, posterior quarter flared in dorsal view, anterior portion slightly broadening in dorsal view, no cornuti evident; eighth sternite almost triangular, narrowing posteriorly.

Female. Unknown.

Type material.—*Holotype*: ♂, ECUADOR: *Morona-Santiago*, Río Abanico, km. 20 Macas-Nueva de Octubre rd., 1600 m, 2°8.0'S, 78°12.0'W, 1 Nov 1996 (K. R. Willmott) (FLMNH).

Paratypes: ECUADOR: *Morona-Santiago*, 1 ♂: same data as holotype (KWJH) (genitalic vial KW-07-01); 2 ♂: same locality data as holotype, 26 Oct 2007 (K. R. Willmott) (MECN, FLMNH). *Napo*, 1 ♂: km. 49 Tena-Loreto rd., 1300 m, 0°43.40'S, 77°43.50'W, 18 Mar 2004 (J. P. W. Hall) (KWJH) (molecular voucher KW-070605-01).

Etymology.—This species is named for KRW's father-in-law, James Kenneth Robinson, a good friend who has instilled his passion for nature and wild places in his daughter and grandson.

Distribution, habitat and ecology.—This species is known to date only from eastern Ecuador, where it occurs in Napo, Morona-Santiago and very probably Zamora-Chinchipe provinces (Fig. 4). Individuals apparently identical to this species were observed by KRW on a ridgetop above Zamora, exhibiting perching behaviour similar to that of males at other sites (see below), and almost certainly represent this species. The wide distribution of *A. kennethi* within Ecuador suggests that it should almost certainly occur in northern Peru in addition to southeastern Colombia. The species has been recorded in a narrow elevational band between 1300 and 1600 m in montane forest, and all known observations have been of males on steep, forested ridgetops. Males were observed perching on the tips of prominent leaves 7–10 m above the ground in light gaps and along wide trails in the

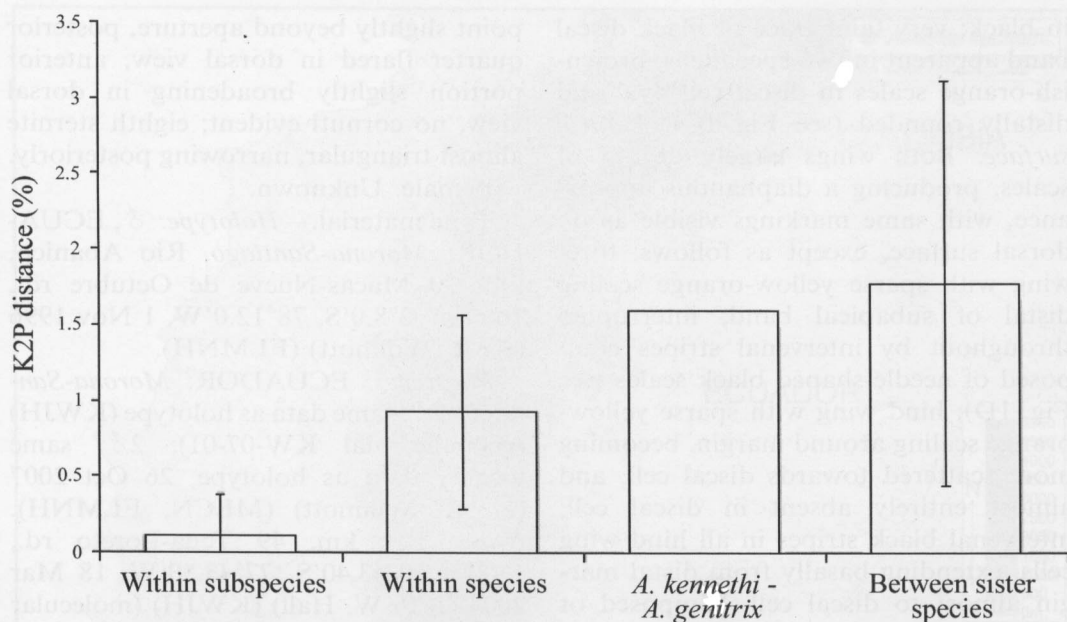


Fig. 5. Mean and range of genetic divergence within species and between sister species of *Actinote* obtained with a K2P substitution model. Divergences were estimated based on the *COI* "barcode" region for all applicable individuals shown in Fig. 3. Bars represent standard deviation.

late morning and early afternoon. They spent much time flying around a particular opening, with a slow, even flight, with few wing beats. The absence of specimens in all collections examined, including the Natural History Museum, London (Huertas personal communication), the Museo Ecuatoriano de Ciencias Naturales, Quito, and the collection of Padre Francisco Piñas, Quito (Piñas 2004), is presumably due to the species' limited and poorly collected elevational range in addition to its high flight.

Discussion.—*Actinote kennethi* apparently forms a clade with *A. g. genitrix* and the recently discovered *A. genitrix costae* from the Sierra de Lema, 1400 m, in the Guiana shield highlands of southeastern Venezuela. This relationship is supported by several morphological characters mentioned in the diagnosis. Furthermore, cladistic analyses of DNA sequence characters (*COI*, *EFlα* and *wingless*) place *A. kennethi* as sister to *A. g. genitrix* (Silva-Brandão *et al.* 2008b; where *A. kennethi* is referred to as "*Actinote* sp2"), as does a

neighbor joining analysis of the "barcode" region (Fig. 3).

Given the allopatry of these three taxa, and their morphological similarity, the taxonomic rank assigned to them is somewhat arbitrary. Although Neild (2008) described the Venezuelan taxon as a subspecies of *A. genitrix*, there is little evidence to suggest that it is more closely related to *A. g. genitrix* than to the new taxon described here. The only morphological characters distinguishing *A. kennethi* from Brazilian *A. genitrix* are in the wing pattern, and we therefore also considered treating *kennethi* as a subspecies. However, we ultimately decided to treat *A. kennethi* as a distinct species based on its known geographic range being so far removed from that of *A. g. genitrix* in southeastern Brazil (Minas Gerais, Espírito Santo, Rio de Janeiro, São Paulo, Paraná, Santa Catarina and Rio Grande do Sul states) (over 3600 km away) and *A. genitrix costae* in southeastern Venezuela (Bolívar state) (over 1900 km away) (see

Fig. 4). Given the rarity of the new taxa, it is likely that further collecting will expand their known ranges and reduce their relative isolation, but based on the distribution of montane habitats where they occur, the three taxa are still likely to be isolated by at least 1000 km. Such large range disjunctions suggest that the *Actinote* taxa in question have been isolated for relatively long periods of time. This suggestion is consistent with a mtDNA (*COI* "barcode" region) cluster analysis showing *A. kennethi* to be 1.6% divergent from Brazilian *A. genitrix*. This level of divergence is comparable with that of other *Actinote* sister species and is just beyond the upper limit of observed intraspecific differences (Fig. 5).

Information about the still unknown immature stages could help clarify the status of *A. kennethi*, as immature stages have been shown to exhibit important morphological differences in other examples of closely related *Actinote* species (Francini 1989, Francini *et al.* 2004). Clearly, the recent discoveries of two new taxa related to *A. genitrix* far removed from the range of that taxon suggest that further new taxa in this group may yet be found.

ACKNOWLEDGMENTS

We thank Carla Penz and Ronaldo Francini for helpful comments on the manuscript. KRW and JPWH thank the National Geographic Society (Research and Exploration Grant #5751-96), Sigma Xi, and the National Science Foundation (Biodiversity Surveys and Inventories grants #0103746, #0639977, #0639861) for assistance with field expenses; Blanca Huertas for sending information about *Actinote* specimens at the Natural History Museum, London; and the Ministerio del Ambiente (Dirección de Bosques y de Areas Naturales Protegidas) and the Museo Ecuatoriano de Ciencias Naturales, in Quito, for arranging the necessary permits for

research in Ecuador. KLSB acknowledges the Brazilian Conselho Nacional de Desenvolvimento Científico e Tecnológico [CNPq] (fellowship #151004/2005-6) and the Fundação de Amparo à Pesquisa do Estado de São Paulo [FAPESP] (grants #06/60127-0, #07/53919-0); and AVLK acknowledges the FAPESP (grants #00/01484-1 and #04/05269-9), the BIOTA-FAPESP program (grant #98/05101-8), the Fundo de Apoio ao Ensino à Pesquisa e à Extensão (PAPDIC grant #1659/04), the CNPq (fellowship #300315/2005-8), and the National Science Foundation (DEB grant #0527441, #0639861).

LITERATURE CITED

- Comstock, J. H. and J. G. Needham. 1918. The wings of insects. *American Naturalist* 32: 253–257.
- Ehrlich, P. R. 1958. The comparative morphology, phylogeny and higher classification of the butterflies (Lepidoptera: Papilionoidea). *The University of Kansas Science Bulletin* 34: 305–370.
- Eliot, J. N. 1973. The higher classification of the Lycaenidae (Lepidoptera): a tentative arrangement. *Bulletin of the British Museum (Natural History) (Entomology)* 28: 373–506.
- Felsenstein, J. 1985. Confidence-limits on phylogenies - an approach using the bootstrap. *Evolution* 39: 783–791.
- Francini, R. B. 1989. *Biologia e ecologia das borboletas Actinote* (Lepidoptera: Nymphalidae, Acraeinae) do sudeste do Brasil. M.S. Thesis, Universidade Estadual de Campinas, Campinas, Brazil.
- Francini, R. B., A. V. L. Freitas, and K. S. Brown, Jr. 2005. Rediscovery of *Actinote zikani* (D'Almeida) (Nymphalidae, Heliconiinae, Acraeini): natural history, population biology and conservation of an endangered butterfly in SE Brazil. *Journal of the Lepidopterists' Society* 59: 134–142.
- Francini, R. B., A. V. L. Freitas, and C. M. Penz. 2004. Two new species of *Actinote* (Lepidoptera, Nymphalidae) from southeastern Brazil. *Zootaxa* 719: 1–10.
- Francini, R. B. and C. M. Penz. 2006. An illustrated key to male *Actinote* from southeastern Brazil (Lepidoptera, Nymphalidae). *Biota Neotropica* Vol. 6.
- Freitas, A. V. L. and K. S. Brown, Jr. 2004. Phylogeny of the Nymphalidae (Lepidoptera). *Systematic Biology* 53: 363–383.

- Klots, A. B. 1956. Lepidoptera, pp. 97–110. In Tuxen, S. L., ed. *Taxonomists' Glossary of Genitalia in Insects*. Munksgaard, Copenhagen, Denmark.
- Kumar, S., K. Tamura, and M. Nei. 2004. MEGA3: integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. *Briefings in Bioinformatics* 5: 150–163.
- Lamas, G. 1996. Lista comentada de los nombres propuestos para los Acraeini neotropicales, y su material-tipo (Lepidoptera: Nymphalidae, Heliconiinae). *Revista Peruana de Entomología* 39: 29–48.
- . 2003. Las Mariposas de Machu Picchu. Guía Ilustrada de las Mariposas del Santuario Histórico Machu Picchu, Cuzco, Peru. PRO-FONANPE, Lima, Peru. 221 pp.
- . ed. 2004. Checklist: Part 4A. Hesperioidea-Papilionoidea. In Heppner, J. B., ed. *Atlas of Neotropical Lepidoptera*. Scientific Publishers, Gainesville, FL, 439 pp.
- Neild, A. F. E. 2008. The Butterflies of Venezuela. Part 2: Nymphalidae II (Acraeinae, Libytheinae, Nymphalinae, Ithomiinae, Morphinae). A Comprehensive Guide to the Identification of Adult Nymphalidae, Papilionidae, and Pieridae. Meridian Publications, London.
- Paluch, M. 2006. Revisão das espécies de *Actinote* Hübner, [1819] (Lepidoptera, Nymphalidae, Heliconiinae, Acraeini). Ph.D. Dissertation, Universidade Federal do Paraná, Curitiba, Brazil.
- Paluch, M., M. M. Casagrande, and O. H. H. Mielke. 2006. Três espécies e duas subespécies novas de *Actinote* Hübner (Nymphalidae, Heliconiinae, Acraeini). *Revista Brasileira de Zoologia* 23: 764–778.
- Penz, C. M. and R. B. Francini. 1996. New species of *Actinote* Hübner (Nymphalidae: Acraeinae) from southeastern Brazil. *Journal of the Lepidopterists' Society* 50: 309–320.
- Penz, C. M. and D. Peggie. 2003. Phylogenetic relationships among Heliconiinae genera based on morphology (Lepidoptera: Nymphalidae). *Systematic Entomology* 28: 451–479.
- Pierre, J. 1987. Systématique cladistique chez les *Acraea* (Lepidoptera, Nymphalidae). *Annales de la Société Entomologique de France* 23: 11–27.
- Piñas, R. F. 2004. Mariposas del Ecuador, Vol. 11a. Familia: Nymphalidae: Subfamilias: Libytheinae, Danainae, Charaxinae, Morphinae, Heliconiinae [sic]. Compañía de Jesús, Quito.
- Saitou, N. and M. Nei. 1987. The Neighbor-Joining Method - a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 4: 406–425.
- Silva-Brandão, K. L., A. M. L. Azeredo-Espin, and A. V. L. Freitas. 2008a. New evidence on the systematic and phylogenetic position of *Parides burchellanus* (Lepidoptera: Papilionidae). *Molecular Ecology Notes*. doi: 10.1111/j.1471-8286.2007.02022.x
- Silva-Brandão, K. L., N. Wahlberg, R. B. Francini, A. M. L. Azeredo-Espin, K. S. Brown, Jr., M. Paluch, D. C. Lees, and A. V. L. Freitas. 2008b. Phylogenetic relationships of butterflies of the tribe Acraeini (Lepidoptera, Nymphalidae, Heliconiinae) and the evolution of host plant use. *Molecular Phylogenetics and Evolution* 46: 515–531. doi:10.1016/j.ympev.2007.11.024.