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DNA BARCODES REVEAL YET ANOTHER NEW SPECIES OF *VENADA* (LEPIDOPTERA: HESPERIIDAE) IN NORTHWESTERN COSTA RICA

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Abstract.—DNA barcodes provided the first clue to ***Venada lamella*** **Burns, new species.** This is the fifth species of the panneotropical but formerly monotypic genus *Venada* to be discovered on Volcán Cacao, a small volcano in Area de Conservación Guanacaste (ACG) in northwestern Costa Rica. Two adult females of this skipper butterfly were reared from caterpillars feeding on a species of *Ocotea* (Lauraceae). The skipper's ACG congeners have been reared in large numbers and found to eat plants not only in *Ocotea* but also in five other lauraceous genera. One adult male of *V. lamella* was taken at black light on adjacent Volcán Orosí. Barcodes clearly associate the sexes and widely separate all five species of *Venada* in a neighbor-joining tree. Interspecific barcode differences range from about 4.5% to about 8.3%. Traditional taxonomic characters (facies, male and female genitalia) distinguish *V. lamella* from its congeners and reinforce the barcode data. Since *V. lamella* inhabits rainforest, and its congeners in ACG inhabit rainforest and cloud forest, and since genus *Venada* is panneotropical, it probably comprises many more species than are currently recognized. Although five species have been described from a limited area within ACG, their geographic distributions are undoubtedly more extensive.

Key Words: genitalia (male and female), foodplants, Lauraceae, *Ocotea hartshorniana*, Area de Conservación Guanacaste, variation, caterpillar

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Even genera in relatively well-known groups of animals may have many more species than we think. Nowadays, DNA barcodes often provide first clues to undetected species. If possible, description of such species should combine the limited molecular data with data derived from independent covarying characters.

Conversely, barcodes are helpful when they support conclusions already evident from classic taxonomic characters. Introduced as a genus with a single panneotropical species, *Venada* (Evans 1952) remained monotypic until the description of four similar species from a total of 327 adult skipper butterflies reared from 636

caterpillars and pupae, all collected on a small volcano in Area de Conservación Guanacaste (ACG) in northwestern Costa Rica (Burns and Janzen 2005). The original descriptions of those species used various characters of morphology (adult and larval facies, male and female genitalia) and ecology. Because subsequent barcoding distinguished the species unequivocally, barcodes alone could be used in the future for specific identification.

Given multiple species of *Venada* at just one point within its wide geographic range (Mexico to Bolivia), Burns and Janzen (2005) predicted the existence of a substantial number of additional species. To our surprise, however, barcodes now indicate a fifth species of *Venada* on the very same Costa Rican volcano, Volcán Cacao, as well as on adjacent Volcán Orosí, to which Cacao connects. We now anticipate numerous species in the Neotropics as a whole.

Materials and Methods

For collecting and rearing procedures connected with inventory of lepidopteran biodiversity in ACG, see Burns and Janzen (2001) and Janzen et al. (2009). For the system of designating wing veins, see Nielsen and Common (1991) and Ackery et al. (1999); wing cells are defined by the veins that flank them. For techniques of genitalic dissection, see Burns (1964); stereomicroscopes used in dissection and study of genitalia were a Wild M5-53355 and a Leitz widefield model TS, respectively, and their associated light sources were Bunton DDL and Intralux 5000. Photographic images were made using the Visionary Digital (TM) BK Lab system. For the adults, the Canon 7D was outfitted with a 100 mm Canon macro-lens, a single photograph taken and then post-processed in Adobe Photoshop. Images of the signa were made using the Infinity Optics K2 Long Distance Microscope, coupled with the CF4 lens. A series of photographs were taken, these were then stacked as layers and post-processed manually in Adobe Photoshop to create a single focused image. For methods used in DNA barcoding, see Hajibabaei et al. (2006) and deWaard et al. (2008). GenBank codes for accessing the results are DQ293856–DQ293878, GU150141–GU150149, GU156725, HM424366–HM424369, HM905347, HQ963911, JF751917, JF754386–JF754388, JF761298–JF761317, JF763457–JF763481, JF778604–JF778608, JQ523137–JQ523142, JQ526765, JQ532495, and JQ546132.

Results

Venada lamella Burns, new species

Description.—*Size:* Forewing (FW) length of one male 24.5 mm; of females 21.7 mm and 22.8 mm. (In skippers, mean FW length of females usually exceeds that of males. The above discrepancy probably reflects the fact that the male was wild-caught whereas the females were reared, and conditions and duration of rearing often stunt growth.)

Antennae: Nudum of club very light brown; number of segments in left nudum/right nudum of two females 30/31 and 31/31. Antennal shaft dark brown with yellow checkering.

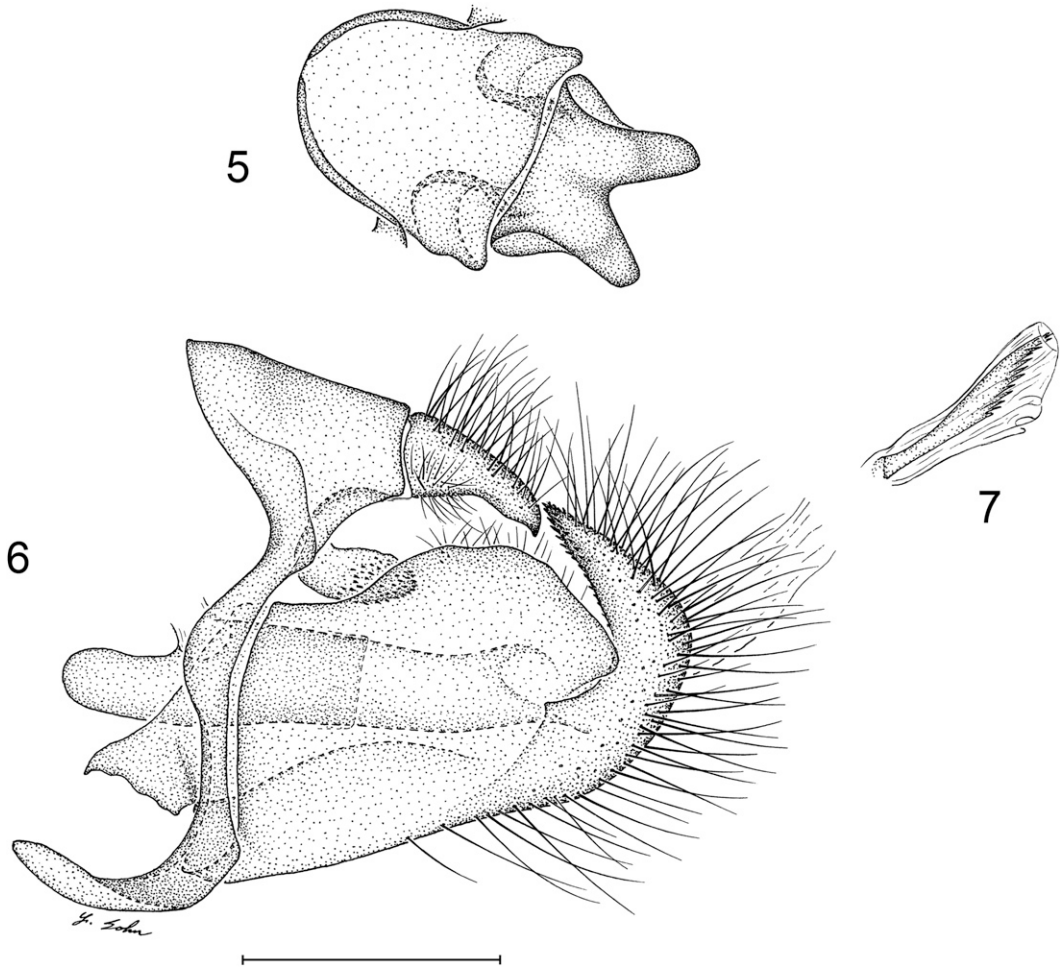
Wing shape (Figs. 1–4): Sexually dimorphic, with FW and especially hindwing (HW) of male longer and narrower than those of female. Male with FW costal fold. In both sexes, tornus of HW lobed.

Facies (Figs. 1–4): Body and wings medium brown, with inconspicuous yellow overscaling. Cheeks almost all brown, but narrowly white along posterior margin

Figs. 1–4. Reared holotype female (above) and wild-caught paratype male (below) of *Venada lamella* in dorsal (left) and ventral (right) view. Pins and pinholes artificially removed. Female photographed before removal of abdomen for genitalia dissection.

of eye. FW with median, wide, oblique, light yellow hyaline band, comprising four conjoined spots and extending from anterior edge of discal cell to middle of cell CuA_2–1A+2A. Spot in discal cell large, spot in cell CuA_1–CuA_2 larger, and both spots roughly rectangular. Spots in cell M_3–CuA_1 and cell CuA_2–1A+2A smaller and triangular, the former on its side and pointing inward, the latter inverted and pointing downward. Distal edge of band nearly or quite even, with anterior point of triangular spot in cell M_3–CuA_1 close to posterior, distal corner of discal cell spot. Male with tiny, light yellow hyaline spot in cell Sc–R_1 barely touching anterior, distal corner of discal cell spot; female with pair of light yellow hyaline spots, somewhat resembling tiny = sign, in costal cell and cell Sc–R_1, and more or less centered over discal cell spot. Both sexes with tiny, white hyaline, subapical spots in cells R_3–R_4 and R_5–M_1, and male with another in cell R_2–R_3. Ventral HW with narrow parallel bands (darker brown on medium brown ground color) about 1/3 and 2/3 out from base of wing and extending from vein Sc+R to vein 1A+2A; distal edge of distal band faintly outlined with light scales. Bands (mainly distal one) visible dorsally but more weakly expressed.

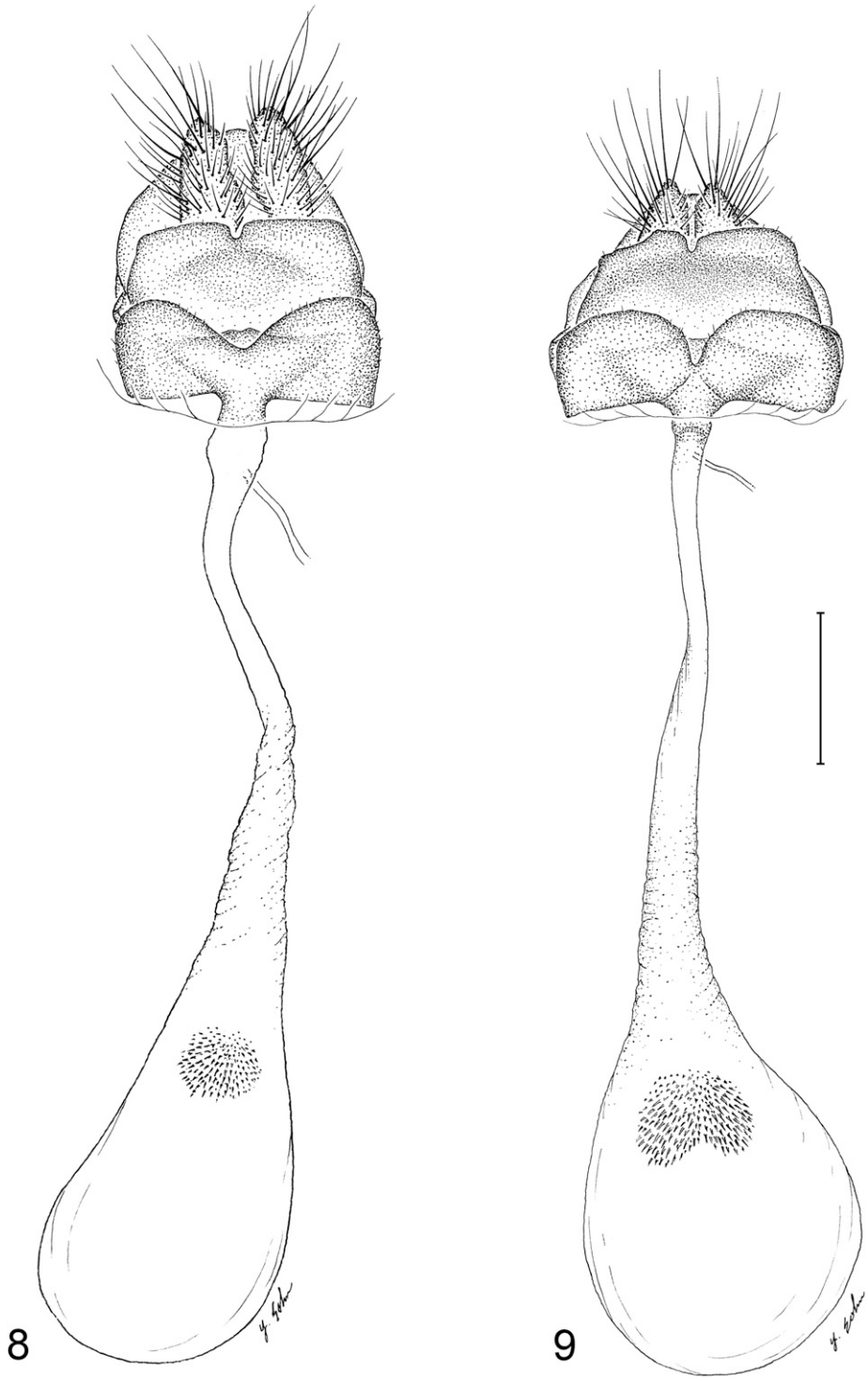
Genitalia: Male (Figs. 5–7): In lateral view, distal, dorsally directed process of valva evenly curved along distal margin, evenly tapered to sharp point, dentate (mostly along straight proximal margin), and narrowly separated from body of



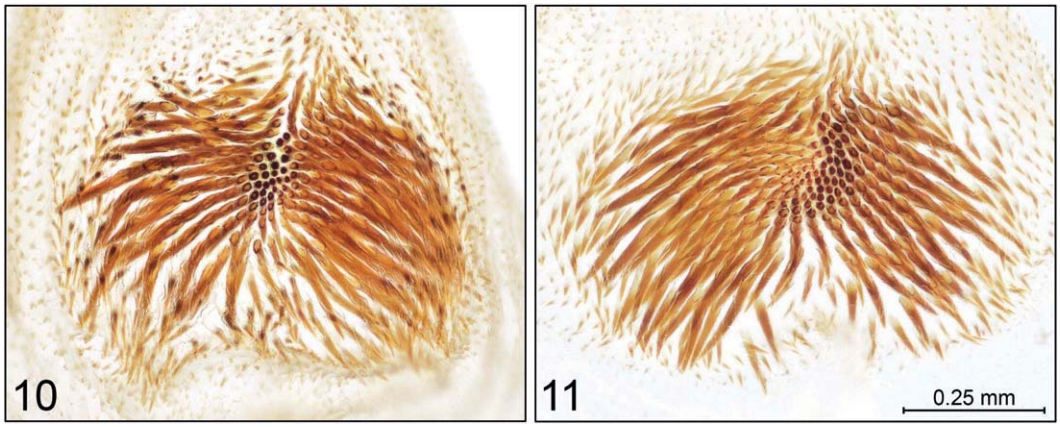
Figs. 5–7. Male genitalia of paratype of *Venada lamella*; J. M. Burns genitalia dissection X-6974; scale bar = 1.0 mm. 5, Tegumen and uncus in dorsal view. 6, Complete genitalia (minus right valva) in left lateral view. 7, Cornutus, within part of vesica.

valva for most of its length; base of process less than half its length; viewed from in front, dorsal half of dentate proximal margin flattened, forming two dentate edges (instead of one). Distal 4/5 of body of valva prominently and evenly humped. Distal half of uncus widely **V**-shaped in dorsal view. Entrance to phallus not extremely close to proximal end of phallus. Cornutus narrow, with distal half resembling a comb.

Female (Figs. 8–11): In ventral view, lateral plates of lamella antevaginalis wide and in contact, or fully joined, midventrally and as long (anterior to posterior) as, or longer than, exposed part of lamella postvaginalis. Small, exposed part of central piece of lamella antevaginalis inconspicuous and about flat. Union of ductus seminalis with ductus bursae dorsal, and near posterior end of ductus bursae. Posterior end of corpus bursae with rounded signum comprising many short, somewhat overlapping and aligned spines directed outward and anteriad from anteriorly directed central



Figs. 8–9. Female genitalia of *Venada lamella* in ventral view; scale bar = 1.0 mm. 8, Holotype, genitalia dissection X-6944. 9, Paratype, genitalia dissection X-6943.



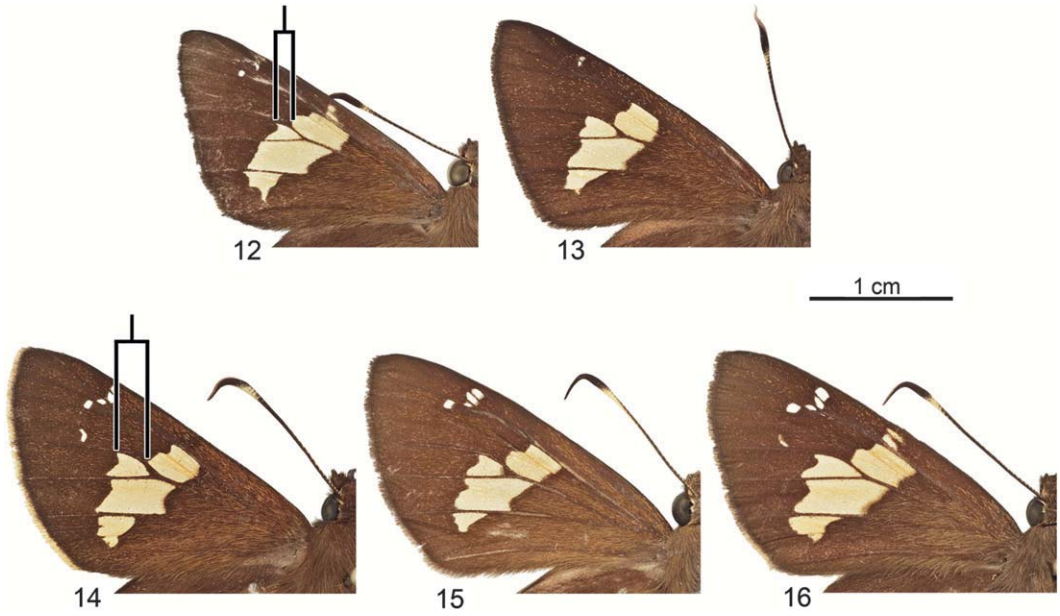
Figs. 10–11. Signum of female genitalia of specimens in Figs. 8–9. Both photographs at the same scale.

spines and forming more or less bilaterally symmetric pattern.

Larval foodplant: Ocotea hartshorniana Hammel (Lauraceae). Five caterpillars found feeding on mature leaves in the flowering crown of a grown (15 m tall) specimen of this tree. Two caterpillars died of disease and one from parasitization by an undescribed species of *Apanteles* (Microgastrinae, Braconidae); the other two caterpillars produced adult females.

Type material.—*Holotype:* female (Figs. 1–2, voucher code 09-SRNP-36371, Naranjales, Sector Cacao, Area de Conservación Guanacaste, Guanacaste, Costa Rica, 1030 m, latitude 10.92268, longitude -85.46405; collected as caterpillar by Manuel Pereira on 06/22/2009; adult eclosed 08/17/2009. Deposited in National Museum of Natural History, Smithsonian Institution (USNM). J. M. Burns genitalia dissection code X-6944. Yellow label reads "LEGS AWAY/FOR DNA." DNA barcode (comprising 658 nucleotides): AACTTTATATTTTATTTTTGGAATTT GAGCAGGACTAGTAGGAACATCAT TAAGATTACTAATTCGTACTGAA TTAGGTACCCCCGGATCTTTAATTG GTGATGATCAAATTTATAATACTATT GTAACAGCTCATGCTTTTATTATAA TTTTTTTTATAGTTATACCTATTATA ATTGGAGGATTCGGAAATTGATTA GTACCTCTTATATTAGGAGCTCCTG ACATAGCATTCCCTCGTATAAATAA TATAAGATTTTGATTATTACCTCCAT CATTAACTCTTTTAATTTCAAGAAG TATTGTCGAAAATGGTGCTGGAAC AGGATGAACAGTATACCCCCCTCTT TCAACAAATATTGCTCATCAAGGA GCTTCTGTAGATTTAGCAATTTTTT CCCTTCACTTAGCAGGTATTTC ATC TATTCTTGGAGCTATTAATTTTATTA CAACAATTATTAATATACGAATTAA TAATTT ATCATTCGATCAAATACCT TTATTTATTTGAGCTGTTGGAATTA CAGCATTATTATTATTACTTTCTTT ACCTGTTTTAGCTGGAGCTATTACA ATATTATTAACAGATCGAAATTTAAA TACCTCATTTTTTGATCCTGCAGGA GGAGGAGATCCTATTTTATACCAAC ATTTATTT.

Paratypes: 1 female, voucher code 09-SRNP-36370, locality and collection data as for holotype; adult eclosed 08/12/2009. Deposited in USNM. Genitalia dissection code X-6943. Yellow label reads "LEGS AWAY/FOR DNA." 1 male (Figs. 3–4), voucher code 11-SRNP-102674, Sendero Memo, Sector Pitilla,



Figs. 12–16. Forewings of females (dorsal view) of the five species of *Venada* in ACG, Costa Rica, showing how the position of the hyaline spot in cell M_3–CuA_1 relative to the hyaline spot in the discal cell clearly distinguishes *V. lamella* from its congeners. Pin holes artificially removed. 12, *V. lamella*, holotype, voucher code 09-SRNP-36371. 13, *V. cacao*, 02-SRNP-23004. 14, *V. naranja*, 04-SRNP-36053. 15, *V. daneva*, 04-SRNP-55125. 16, *V. nevada*, 02-SRNP-9426.

Area de Conservación Guanacaste, Guanacaste, Costa Rica, 774 m, latitude 10.98518, longitude -85.42811; collected at black light by R. Franco and S. Rios on 04/04/2011. Deposited in USNM. Genitalia dissection code X-6974. Yellow label reads "LEGS AWAY/FOR DNA."

Etymology.—The specific name *lamella* refers to the highly distinctive lamella antevaginalis of the female genitalia and continues the practice of giving three-syllable, euphonius names, accented on the middle syllable, to species of *Venada*.

Diagnosis.—A conspicuous feature of the large, yellow hyaline band of the FW is as diagnostic as are DNA barcodes: in both sexes of *V. lamella*, the anterior point of the triangular spot in cell M_3–CuA_1 is very close to the lower, outer corner of the spot in the discal cell, so that the distance between these points is far shorter than it is in other named species of *Venada* (Figs. 12–16). Helpful in narrowing the choice of species (but not definitive) are the almost entirely dark cheeks of *V. lamella*, which distinguish it from the white-cheeked species, *V. nevada* Burns, *V. daneva* Burns, and *V. advena* (Mabille). In the female genitalia, the midventral contact or union of the expanded lateral divisions of the lamella antevaginalis is obvious and unique to *V. lamella*. The distinctive, roughly bilaterally symmetric form of the signum is shared only with *V. cacao* Burns. In the male genitalia, where differences are more subtle, what best sets *V. lamella* apart from congeners is a combination of characters in the distal, dorsally directed process of its valva, i.e., even curvature of the posterior margin, a short base, and a dentate distal portion that is long but not narrow for most of its length.

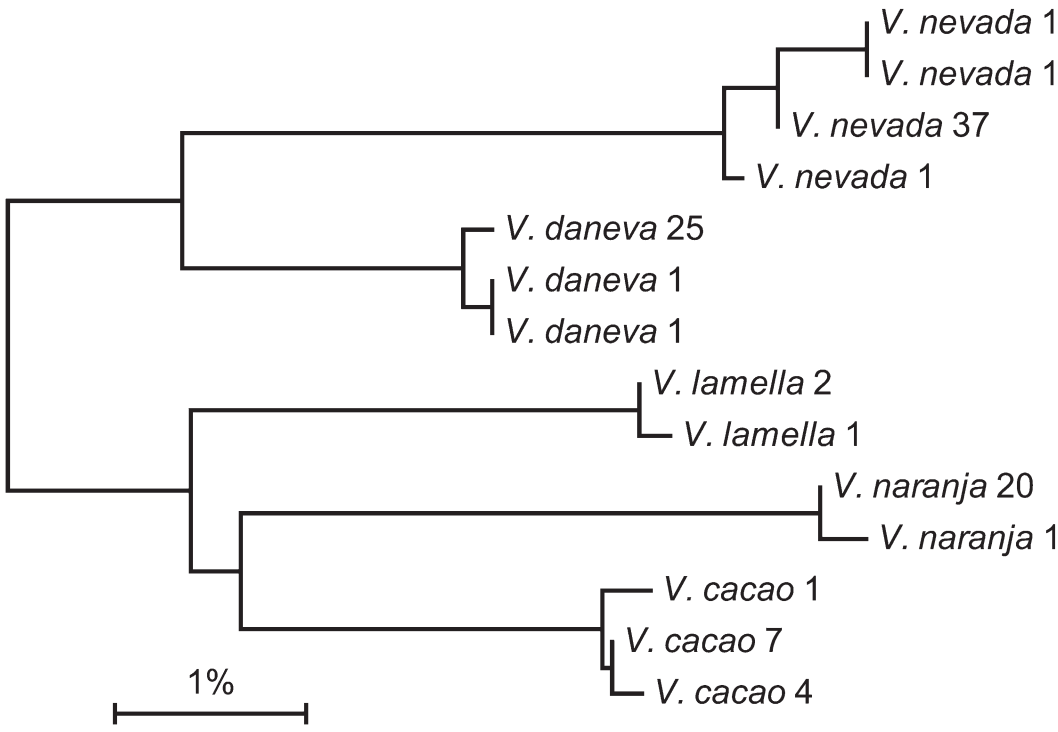


Fig. 17. Neighbor-joining tree based on Kimura two-parameter distances for CO1 DNA barcodes of the five species of *Venada* reared in ACG, Costa Rica. Number of individuals with each haplotype on the right.

Discussion

Although both females of *V. lamella* are reared from caterpillars collected in one tree and the lone male is a wild-caught adult found about 15 km away, barcodes clearly associate the sexes. *Venada* barcodes are intraspecifically conservative (Fig. 17). Interspecific barcode differences in *Venada* are unquestionably diagnostic, varying from ca. 4.5% to ca. 8.3%, which is an ordinary range for noncryptic congeners (Janzen et al. 2011). The barcode difference (ca. 4.5%) between *V. lamella* and *V. cacao* is as small as the difference (ca. 4.7%) between *V. nevada* and *V. daneva*. These differences lie at the low end of the *Venada* range (Fig. 17) but are nonetheless large. In rare instances, interspecific barcode differences are very much smaller. For example, barcodes of three of the four cryptic but indisputable species of skippers in the *Perichares* complex in ACG differ by only ca. 0.6% to ca. 0.8% (Burns et al. 2008). A still rarer case involves a few skipper species whose barcodes can differ by just 1–3 nucleotides (Burns et al. 2007).

Description of *V. cacao*, *V. naranja* Burns, *V. daneva*, and *V. nevada* from samples of 5, 19, 69, and 234 adults, respectively, treated individual variation in facies (Burns and Janzen 2005). Expression of the small to tiny costal and subapical FW spots is taxonomically significant but variable. Intraspecific expression of these hyaline spots varies both between and within the sexes so that, in some cases, interspecific differences are merely average and quite

small. Given but one male and two females of *V. lamella*, their apparent reduced expression of subapical spots compared with other species of *Venada* may be minimally relevant in distinguishing the species.

Adults of the known species of *Venada* have similar facies, but their caterpillars do not: *V. nevada*, *V. daneva*, and *V. cacao* differ greatly from one another; and *V. cacao* and *V. naranja*, despite their resemblance, differ enough to be readily distinguished (Burns and Janzen 2005: figs. 30–33). However, in rearing *V. lamella*, the parataxonomists neither noticed nor recorded any larval differences, assigning the caterpillars to *V. cacao* in the field when they were collected.

Unlike many coexisting congeneric species of skippers with major differences in their choice of larval food, the species of *Venada* in ACG feed on plants in six genera of one family, Lauraceae: *Beilschmiedia*, *Licaria*, *Nectandra*, *Ocotea*, *Persea*, and *Pleurothyrium* (Burns and Janzen 2005, Janzen and Hallwachs 2012). However, despite collective conservatism and appreciable overlap in foodplant choices, each species of *Venada* has a unique pattern of foodplant preferences—except for *V. lamella*: *O. hartshorniana*, the only known foodplant of *V. lamella*, is also eaten by *V. cacao*, along with other species of *Ocotea* and species in three other genera of Lauraceae. A distinctive pattern in *V. lamella*'s foodplant selection may emerge when more caterpillars are found.

The four species of *Venada* previously described from ACG inhabit cloud forest and rainforest (Burns and Janzen 2005). Meager data indicate that *V. lamella* is another rainforest species. The localities at which it has been taken are about 15 km apart. Although the two reared females come from an elevation of 1030 m, their exact collection site is at the lower margin of the clouds and warmer than the high elevation cloud forest. The wild-caught male comes from an elevation of 774 m, to which clouds rarely descend. Despite the small sample, *V. lamella* clearly coexists with *V. nevada* and *V. daneva*. It may be parapatric with the cloud forest species, *V. cacao* and *V. naranja*.

In contrast to its ecologic distribution, the geographic distribution of *V. lamella* is unknown. The intense and lengthy bioinventory of Lepidoptera in ACG (Janzen and Hallwachs 2012) has resulted in description of many new species solely from specimens collected in ACG. This does not mean that those species have such a limited range.

The neighbor-joining tree derived from barcodes (Fig. 17) splits the ACG species of *Venada* into two groups: a *daneva* group, comprising *V. daneva* and *V. nevada*, and a *cacao* group, comprising *V. cacao*, *V. lamella*, and *V. naranja*. Various nonmolecular characters covary with the barcodes. In the *daneva* group as opposed to the *cacao* group, the ground color of the wings is a paler brown; and the cheeks of the head are white instead of mostly dark. Although the caterpillars of *V. daneva* and *V. nevada* differ greatly in their bold abdominal spotting, they resemble each other far more than they do the caterpillars of *V. cacao* and *V. naranja*, whose paler heads lack eyespots and whose bodies are finely dotted with white (Burns and Janzen 2005: figs. 30–33). Similarity of the latter two species extends to three paired, large, round, bright yellow, abdominal spots; and those of *V. cacao* are accentuated by peripheral black, not present in *V. naranja*. Because parataxonomists mistook the caterpillars of *V. lamella* for those of *V. cacao*, the caterpillars of all three species in the *cacao* group are presumably very simi-

lar. Little more can be said of groupings without knowledge of some of the anticipated undescribed species of *Venada*.

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