

Science**Hidden Neotropical Diversity: Greater Than the Sum of Its Parts**Marty A. Condon, *et al.*
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Movie S1

Data Set S1

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Hidden Neotropical Diversity: Greater Than the Sum of Its Parts

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The diversity of tropical herbivorous insects has been explained as a direct function of plant species diversity. Testing that explanation, we reared 2857 flies from flowers and seeds of 24 species of plants from 34 neotropical sites. Samples yielded 52 morphologically similar species of flies and documented highly conserved patterns of specificity to host taxa and host parts. Widespread species of plants can support 13 species of flies. Within single populations of plants, we typically found one or more fly species specific to female flowers and multiple specialists on male flowers. We suggest that neotropical herbivorous insect diversity is not simply a function of plant taxonomic and architectural diversity, but also reflects the geographic distribution of hosts and the age and area of the neotropics.

The diversity of neotropical herbivorous insects, ranging in number from 3 million to 30 million species (1), has been hypothesized to be a function of plant diversity (2, 3), but the degree to which specialization shapes that function is contentious. Plant architecture (4, 5) and distribution also affect patterns of insect diversity (6, 7). Diversity estimates have traditionally been generated from counts of morphologically distinguishable insect species (morphospecies) collected on plant surfaces (2, 3). However, molecular evidence suggests that tallies of morphospecies underestimate both diversity and host specificity (8, 9). Temperate zone research has revealed diverse assemblages of host-specific cryptic species as well as recently diverged host races (10), including groups that diversified to different parts (e.g., leaves, flowers, stems) of the same host plant (11). Although

different plant tissues represent numerous niches in the tropics, few studies have assessed the diversity of concealed larvae feeding inside those parts (12, 13), and even fewer have used molecular markers to reveal cryptic species (14).

To address the relationship between host and insect diversity, we focused on *Blepharoneura* (15), a neotropical genus of tephritid fruit flies that, as larvae, feed within the flowers or fruits of plants in the cucumber family (Cucurbitaceae). *Blepharoneura* larvae rarely cause external signs of damage, and few host records existed prior to this study. More than half of the known host species of this group of *Blepharoneura* belong to the Guraniinae, a cucumber subtribe characterized by architectural complexity. The two largest genera within the Guraniinae (*Gurania* and *Psiguria*) have brightly colored flowers with succulent outer floral organs (calyces), typical of many hummingbird-pollinated plants (fig. S1). Most species have male and female flowers borne on sexually dimorphic branches, which are temporally and spatially isolated on individual plants (16). Because only large plants produce female branches, which produce fewer flowers for a shorter period of time than male inflorescences, female flowers are rarer than male flowers. Thus, a population of a single host species represents a mosaic of morphologically distinctive

targets differing in abundance at any point in time (fig. S2).

To investigate patterns of host use and diversity in *Blepharoneura*, we reared 2857 flies from 24 different cucurbit host species in nine genera and three tribes. Our sample encompassed 10 distinct biogeographic neotropical regions (table S1) spanning the geographic distribution of the subtribe Guraniinae, from Mexico to southern Bolivia (~5500 km) and from the Pacific to the Atlantic coasts of South America (~3000 km). We analyzed 419 specimens from 34 sites in 10 countries. With a conservative 4% sequence divergence cutoff for species limits (15), a phylogenetic analysis of mitochondrial cytochrome c oxidase subunit I (mtCOI) sequence revealed 52 species of flies (figs. S3 to S8). Most of these species were morphologically indistinguishable (15) but had sequence differences ranging from 6 to 18% (Fig. 1). Because divergent groups revealed by mtCOI can incorrectly identify species (17), we examined two nuclear genes from 58 specimens from the Napo region of Ecuador. Analysis of nuclear elongation factor 1- α (EF1- α) and CAD (15) recovered the same 10 lineages (fig. S9) and corroborated the mtDNA results (Fig. 1), which suggests that these lineages indeed represent distinct species. Subsequent morphological analyses of a subset of these genetically defined species revealed slight but statistically significant differences in morphology (18).

Cryptic species of *Blepharoneura* showed specificity both to host part and to host taxon: Of 45 species reared from reproductive tissues, only a single species (sp. 39) fed on both flowers and seeds, whereas all other species appeared to be restricted to either flowers or seeds. Among flower-feeders, most specialized on flowers of a single gender. This is surprising because most flower-feeders feed primarily on calyx tissue, which is similar in flowers of both sexes. Furthermore, many species specialized on female flowers, which are rare relative to male flowers (fig. S2) (15, 16), in contrast to predictions that insects are less likely to specialize on rare hosts (19). Both seed- and flower-feeders tended to

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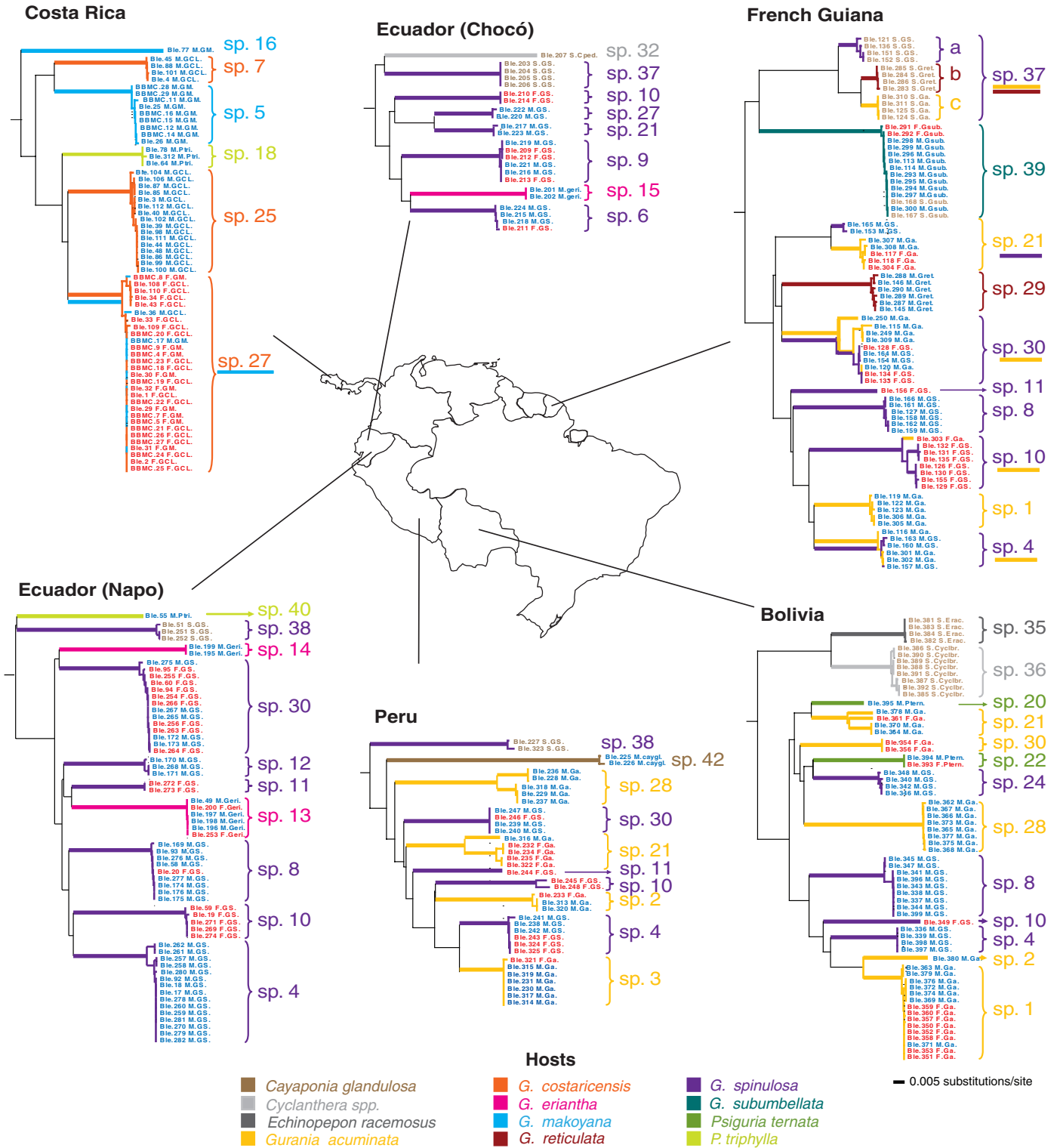


Fig. 1. Multiple sympatric species, often specific to flower gender, infest single species of hosts in diverse geographic areas. Neighbor-joining trees were constructed from mtCOI sequence data from specimens collected in 6 of the 10 biogeographic regions we sampled (15). Branch colors indicate host species (see key). Terminal labels indicate host part infested: red, female flowers; blue, male flowers; tan, seeds. Species are identified with numbers as in figs. S3 to S8; underlined identification numbers indicate species reared from more than one host species; letters (a, b, c) highlight

lineages within species 37 that would be recognized as distinct species if less conservative criteria were used to delineate species (15). See table S1 for locality data for transects (identified by italic letters and numbers): Trees denoted Costa Rica (C14), the Chocó region of northwestern Ecuador (E20), the Napo of eastern Ecuador (E19), and Peru (P31) were constructed from samples collected along single transects (each <20 km long with elevation variation of <400 m); trees denoted French Guiana (F22 to F25) and Bolivia (B1 to B7) were from samples collected along more than one transect.

specialize on a single species; 80% were reared from a single host plant species (15). The proportion of host taxon specialists did not

significantly change when we used less conservative criteria for species delimitation (15). We also found, contrary to predictions (19), that the

rarer host plants in this study supported only host-taxon specialists and were not hosts to generalist species (Fig. 1).

Fig. 2. Maximum likelihood tree of 45 species of *Blepharoneura*. Maximum likelihood analysis used a combined data set of the nuclear CAD and EF1- α genes and the mitochondrial COI gene. Fly species, each represented by a single individual, are identified by numbers (figs. S3 to S8; spp. 44 and 45 lack nuclear sequences and are not included here). Collection localities for each species are indicated by letters: A, Panama; B, Bolivia; C, Costa Rica; E, eastern Ecuador; F, French Guiana; G, Guyana; M, Mexico; P, Peru; V, Venezuela; W, western Ecuador (table S1). Colors of branches and letters indicate most commonly used hosts in an area; colors of rectangles around letters indicate additional hosts at some sites. Flower color indicates host taxon: red, Guraniinae; white, Cucurbitaceae. Host nomenclature is as in appendix S1.

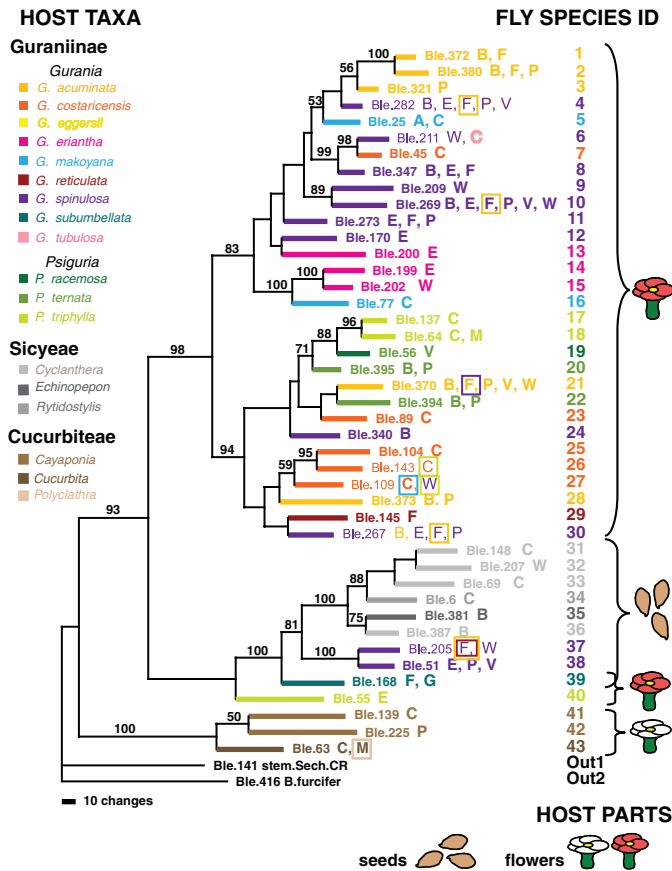
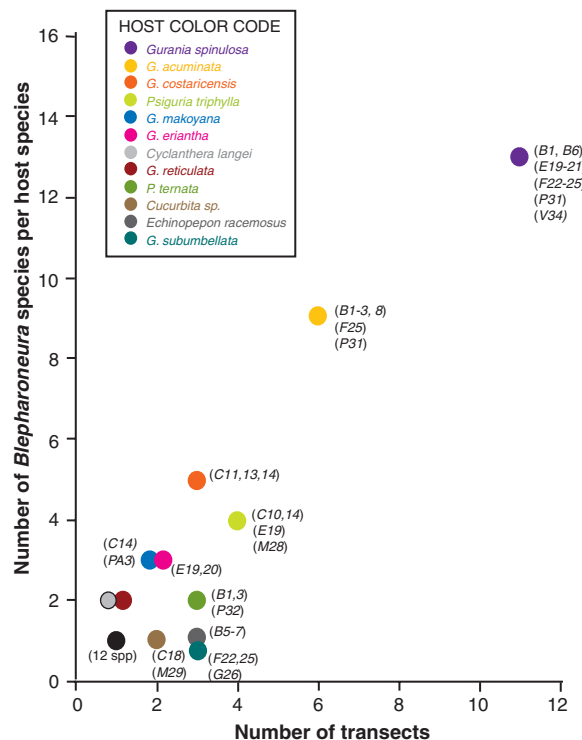


Fig. 3. Diversity of *Blepharoneura* species per host species increases with number of transects. Host plants found in multiple transects host more species of *Blepharoneura* than do rarer species and species with more limited distribution ($r = 0.9337$). Colors (see key) identify hosts; transect identification numbers are listed by country (table S1). All transects are <20 km long with elevation variation of <400 m.



Maximum likelihood analysis of the genetic data revealed distinct groups of seed- and flower-feeding species restricted to specific plant parts and plant taxa (Fig. 2). Two clades of flower-feeders associated with groups characterized by relatively large flowers and succulent tissues. Species 1 to 30 were flower-feeders found on plants within the subtribe Guraniinae (98% bootstrap support). Alternatively, species 41 to 43 were restricted to the tribe Cucurbitaceae (100% bootstrap support). Within the seed-feeding clade (spp. 31 to 39), two strongly supported lineages appeared to be restricted to the subtribe Sicyeae (100% bootstrap support) and the Guraniinae (100% bootstrap support), respectively (Fig. 2). Thus, the number of *Blepharoneura* species reflects the diversity of different plant parts and host taxa but exceeds the sum of plant part types for some plant taxa, because multiple species of flies infest a single type of plant part. We observed that some host species were infested by at least 13 species of *Blepharoneura*. We typically found four to six species infesting flowers in a population of a single species of host (Fig. 1 and table S1).

Our data show that geographically widespread host plants supported more species, both locally and regionally, than did hosts with more limited distributions (Fig. 3), reflecting patterns observed in the temperate zone (6, 7). The most abundant and widespread species of *Gurania* (*G. spinulosa*) was infested by 13 species of *Blepharoneura* (Figs. 1 to 3, table S1, and appendix S1). *Gurania acuminata*, another widespread but locally rare species, hosted nine species of *Blepharoneura*, four of which were reared from flowers collected on a single day from the same tangle of vines in Peru (spp. 2, 3, 21, and 28; Fig. 1). The close ecological associations of sympatric species feeding on the same host taxon and tissue were not unusual; even on less common hosts (e.g., *G. eriantha*), we reared multiple species from single inflorescences (spp. 13 and 14; see Napo, Fig. 1).

More than half of the *Blepharoneura* species we discovered were found at only one site and may have very limited ranges (Figs. 1 and 2, figs. S3 to S8, and table S1). Species endemic to particular regions were not restricted to rare hosts or to hosts with limited geographic distributions. Three of the 13 species infesting the widespread *G. spinulosa* appeared to be local endemics: One species appeared to be restricted to the Napo region of eastern Ecuador (sp. 12; Fig. 2 and fig. S5), one to northwest Ecuador (sp. 9; Fig. 2 and fig. S5), and one to the southernmost limit of *G. spinulosa*'s distribution in Bolivia (sp. 24; Fig. 2 and fig. S3). Most widespread species of *Blepharoneura* showed a high degree of fidelity to host species and tissue, but we detected some geographic variation in host use patterns (e.g., spp. 27 and 30; Fig. 1 and figs. S6 and S7). For example, species 10 was distributed

throughout tropical South America, fed exclusively on female flowers, and, in all but one locality, fed on a single species of host (Fig. 1 and fig. S4). Other species (e.g., sp. 27) fed almost exclusively on female flowers (30 of 32 specimens) of at least two host species in Central America, but commonly fed on male ($N=4$) and female ($N=4$) flowers in areas west of the Andes in Ecuador (Fig. 1 and fig. S6). These variable patterns of host use form a mosaic that varies from community to community across large geographic areas (20) and complicates attempts to extrapolate local samples to global estimates of tropical diversity (21).

Although we report diversity exceeding the original morphological estimates by an order of magnitude (15), this must underrepresent the actual diversity of this group because our criterion for species delimitation is highly conservative (15). This is because we used a 4% mtCOI divergence, whereas other studies recognize species differing by less than 1% (8). As a result of this conservative criterion, we may be lumping biologically distinct species together, and single generalist species may actually represent multiple host-specific species (e.g., sympatric monophyletic lineages feeding on separate hosts; see sp. 37 in French Guiana, Fig. 1 and fig. S8). Also, our samples are limited; most of our collections were made during single trips, and our samples were restricted to species in fruit or flower at that time (table S1). Finally, the number of fly species recorded for a particular host plant species was most likely limited because the number of insect species detected rose as the number of collection localities increased (Fig. 3).

We also found that the distribution of hosts may also predict herbivore diversity at both local and regional scales (6, 7). The neotropics include a mosaic of biogeographic zones reflecting a long history of repeated habitat fragmentation (22). During periods of habitat fragmentation, insect populations may be more likely than these plant populations to diverge, as insects have shorter generation times and can evolve more quickly than plants with long generation times (15). Furthermore, sexual selection accelerates rates of evolution in insects, particularly in groups with complex courtship displays such as *Blepharoneura* (9, 18, 23). When these new species come together, as habitats expand and host populations rejoin, assemblages of highly host-specific cryptic species result. In local assemblages of *Blepharoneura* (Fig. 1), the minimum pairwise divergence among sympatric species is ~6%, which suggests that they diverged at least 2.6 million years ago (24). During the past 2.6 million years, even seemingly uniform habitats experienced multiple cycles of fragmentation and expansion (22). If host plants represent “hard boundaries” (25) for ranges of host-specific insects, simple neutral models incorporating changes in habitat area (25) as well as time (26, 27) could help account for patterns of diversity. Conflicting assessments of host specificity and diversity in the

tropics (2, 3, 28) may reflect differences in geographic scale rather than differences in evolutionary or ecological processes.

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Supporting Online Material

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Tables S1 and S2
Appendices S1 and S2
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Surface Tension Transport of Prey by Feeding Shorebirds: The Capillary Ratchet

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The variability of bird beak morphology reflects diverse foraging strategies. One such feeding mechanism in shorebirds involves surface tension–induced transport of prey in millimetric droplets: By repeatedly opening and closing its beak in a tweezing motion, the bird moves the drop from the tip of its beak to its mouth in a stepwise ratcheting fashion. We have analyzed the subtle physical mechanism responsible for drop transport and demonstrated experimentally that the beak geometry and the dynamics of tweezing may be tuned to optimize transport efficiency. We also highlight the critical dependence of the capillary ratchet on the beak's wetting properties, thus making clear the vulnerability of capillary feeders to surface pollutants.

Phalaropes (Fig. 1A) and several other shorebirds with long thin beaks feed primarily on small crustaceans and other invertebrates (1). By swimming in a tight circle on the water surface, they generate a vortex that draws underlying fluid and suspended prey toward the surface (2). By pecking on the water surface at a rate of ~1.5 Hz (1, 3–6), the birds capture water droplets with a characteristic scale of ~2 mm between their upper and lower mandibles (movie S1). Suction cannot be used to raise the drops mouthward because of the geometry of the open beak; gravity acts to oppose the

drop motion. Nevertheless, the birds succeed in raising the drops mouthward by opening and closing their beaks successively (1, 5, 7, 8). Although the importance of surface tension in this process was inferred (1), the physical mechanism responsible for the droplet transport, specifically

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ERRATUM

Post date 5 September 2008

Reports: "Hidden neotropical diversity: Greater than the sum of its parts" by M. A. Condon *et al.* (16 May, p. 928). The first sentence of the main text included a misplaced reference citation. The sentence should read: "The diversity of neotropical herbivorous insects, ranging in number from 3 million to 30 million species (1), has been hypothesized to be a function of plant diversity (2), but the degree to which specialization shapes that function is contentious (3)."