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Revision of the genus *Reichardtiolus* Kryzhanovskij, 1959 (Coleoptera, Histeridae, Saprininae)

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Abstract

The genus *Reichardtiolus* Kryzhanovskij, 1959 is revised herein. It now contains five species: *R. duriculus* (Reitter, 1904) from middle Asia (with a doubtful female specimen from western China that is here tentatively assigned to this species), *R. pavlovskii* Kryzhanovskij, 1959 from Turkmenistan, *R. sphingis* (Peyerimhoff, 1936), **comb. n.** (transferred from *Saprinus* Erichson, 1834) from Egypt and Jordan, *R. perses* **sp. n.** from Iran and *R. aldhaferi* **sp. n.** from Saudi Arabia. Except for *R. pavlovskii*, which is a rather distinct species known only from two females, the remaining species are allopatric, very similar externally and are best separated from each other by their male terminalia. *R. pavlovskii* is kept in *Reichardtiolus* only tentatively, pending the examination of more specimens, and especially its male genitalia. *R. duriculus* and *R. pavlovskii* are re-described, while *R. perses* **sp. n.**, *R. aldhaferi* **sp. n.** and *R. sphingis* **comb. n.** are provided with diagnostic descriptions because of their overall similarity with *R. duriculus*. Morphological differences of all species are illustrated using SEM micrographs. Male genitalia of *R. duriculus*, *R. sphingis* **comb. n.**, *R. perses* **sp. n.** and *R. aldhaferi* **sp. n.** are illustrated and a key to the species is given. *R. duriculus* is newly recorded from Tajikistan.

Keywords

Coleoptera, Histeridae, Saprininae, *Reichardtiolus*, Palaearctic Region, taxonomic revision

Introduction

The genus *Reichardtiolus* was established by Kryzhanovskij (1959) based on the species *Saprinus duriculus* Reitter, 1904. At the time of its designation *Reichardtiolus* was a mere subgenus of the genus *Exaesiopus* Reichardt, 1926 and Kryzhanovskij (1959) included in it another species, *R. pavlovskii*, which he described in the same work. In their fauna of the USSR, Kryzhanovskij and Reichardt (1976) elevated the rank of *Reichardtiolus* from a subgenus of *Exaesiopus* to fully-fledged genus. Lackner (2010) summarized the knowledge about the genus without having examined the obscure and very rare taxon *R. pavlovskii*. During the years 2006–2013 I had the opportunity to examine a large number of Saprininae taxa, among them the rare *R. pavlovskii* and *Saprinus sphingis* Peyerimhoff, 1936, the latter of which has been treated as a species incertae sedis since its description (Peyerimhoff 1936; Mazur 1984; 1997; 2004; 2011). One undescribed species, apparently belonging to *Reichardtiolus* from Saudi Arabia was recently discovered in the collections of the King Saud Museum of Arthropods (KSMA), and the author's visit to the Zoological Institute of the Russian Academy of Sciences (ZIN) yielded another new species from south-western Iran. The results of these examinations are presented below. This work presents another contribution to the on-going revisionary work of the genera of the subfamily Saprininae (Lackner 2009a-c, 2010, 2011a,b; Tishechkin and Lackner 2012; Lackner 2012; Lackner 2013a,b; Lackner and Gomy 2013).

Material and methods

All dry-mounted specimens were relaxed in warm water for several hours or overnight, depending on the body size. After removal from original cards, the beetles were side-mounted on triangular points and observed under a Nikon 102 stereoscopic microscope with diffused light. Body structures were studied using methods described by Ôhara (1994): male genitalia were macerated in a hot 10% KOH solution for about 15 minutes, cleared in 80% alcohol, macerated in lactic acid with fuchsine, incubated at 60°C for two hours, and subsequently transferred into a 1:1 mixture of glacial acetic acid and methyl salicylate, heated at 60°C for 15 minutes and cleared in xylene. Specimens were then observed in α-terpineol in a small glass dish. Digital photographs of the male terminalia were taken by a Nikon 4500 Coolpix camera and edited in Adobe Photoshop CS4. Based on the photographs or direct observations, the genitalia were drawn using a light-box Hakuba klv-7000. SEM photographs of *R. duriculus*, *R. pavlovskii* and *R. sphingis* were taken with a JSM 6301F microscope at the laboratory of Faculty of Agriculture, Hokkaido University, Sapporo, Japan while those of *R. aldhaferi* and *R. perses* were taken at the Laboratory of the Electron Microscopy at the Faculty of Biology, Charles University, Prague, Czech Republic. All available specimens were measured with an ocular micrometer. Beetle terminology follows that of Ôhara (1994) and Lackner (2010). Separate lines of the same

label are demarcated by a slash (/). The following acronyms of museums and private collections are used throughout the text:

CAS Alexander Sokolov collection, Moscow, Russia;
CAT Alexey K. Tishechkin collection, Baton Rouge, Louisiana, USA;
CND Nicolas Dégallier collection, Paris, France;
CPV Pierpaolo Vienna collection, Venice, Italy;
CYG Yves Gomy collection, Nevers, France;
FMNH Field Museum of Natural History, Chicago, USA (J. Boone);
HNHM Hungarian Natural History Museum, Budapest, Hungary (O. Merkl);
KSMA King Saud Museum of Arthropods , Riyadh, Saudi Arabia (H. M. Al Dhafer);
MSNG Museo Civico di Storia Naturale "Giacomo Doria", Genoa, Italy (M. Tavano);
TLAN Tomáš Lackner collection, temporarily housed at Naturalis Biodiversity Centre, Leiden, Netherlands;
ZIN Zoological Institute, Russian Academy of Sciences, St. Petersburg, Russia (B. Kataev).

Abbreviations. Abbreviations of morphological measurements follow Ôhara (1994) and are used throughout the text as follows:

APW width between anterior angles of pronotum
EL length of elytron along elytral suture
EW maximum width between outer margins of elytra
PEL length between anterior angles of pronotum and apices of elytra
PPW width between posterior angles of pronotum.

Taxonomy

***Reichardtiolus* Kryzhanovskij, 1959**
http://species-id.net/wiki/Reichardtiolus

Reichardtiolus Kryzhanovskij, 1959: 217 (as a subgenus of *Exaesiopus*). Type species *Saprinus duriculus* Reitter, 1904, original designation.
Reichardtiolus: Kryzhanovskij and Reichardt (1976): 112, 238; Mazur (1984): 103; Mazur (1997): 265; Mazur (2004): 96; Lackner (2010): 63, 186; Mazur (2011): 210.

Diagnosis. *Reichardtiolus* has been recently diagnosed by Lackner (2010), but the published diagnosis has to be adapted with respect to the newly examined *R. pavlovskii*, *R. sphingis*, *R. perses* and *R. aldhaferi* as follows: body size 2.00–4.25 mm, cuticle (Fig. 1) chestnut brown to almost black with or without slight metallic tinge or lustre; frontal stria (Figs 2, 3) usually weakened medially, but may be complete to widely interrupted (in *R. pavlovskii*); frons variously densely punctuate, punctures separated by less than

half their diameter to twice their diameter; occasionally with protuberances or shallow depressions; clypeus rectangular to rounded, occasionally margined, anterior margin may be elevated; dorsal surface densely to very densely and coarsely punctuate, punctures separated by their own to half their own diameter, in *R. pavlovskii* even forming longitudinal wrinkles on pronotum (Fig. 62); pronotal depressions absent; dorsal elytral striae in *R. pavlovskii* almost unrecognizable beneath coarse punctuation, in other congeners usually all four dorsal elytral striae 1–4 well discernible; prosternal foveae present (Fig. 10) or absent (*R. pavlovskii*; Fig. 68); prosternal process often compressed, concave or convex, especially on posterior half, punctate and setose; both sets of prosternal striae present (in case of *R. pavlovskii* only as vague rudiments); pronotal hypomeron, lateral disc of metaventrite and metepisternum setose. Protibia (Figs 1, 64) with two or three short teeth each topped by variably large denticle, usually followed by one or two much smaller denticles entombed in outer margin of protibia; meso- and metafemora strongly thickened (Fig. 63); metatibia dilated and thickened; anterior surface of metatibia with two to several rows of short, stout denticles (Fig. 71).

Differential diagnosis. Members of *Reichardtiolus* are externally most similar to the species of the genus *Exaesiopus* Reichardt, 1926, differing from them especially by the absence of deep longitudinal rugae on the frontal disc. The elytra in *Reichardtiolus* are entirely coarsely and densely punctate, in *R. pavlovskii* even forming rugulose-lacunose wrinkles, whereas in *Exaesiopus* the elytra are always at least partly glabrous. Because of the thickened hind femora and lack of longitudinal furrows on frons, *Reichardtiolus* cannot be confused with any other Palaearctic taxon; for further details on differential diagnosis and a key to genera of the Palaearctic Histeridae the reader is referred to Lackner (2010).

Biology. *Reichardtiolus* is a psammophilous taxon, found in arid and desert habitats, often in sand or under decaying vegetation (Lackner 2010); several specimens of *R. aldhaferi* and *R. duriculus* were also collected at light or in rodent's burrows. According to Kryzhanovskij in Kryzhanovskij and Reichardt (1976) the second known specimen of *R. pavlovskii* was collected while digging in sands under *Tamarix*.

Distribution. *R. duriculus* is found across middle Asia: Kazakhstan, Turkmenistan, Uzbekistan and Tajikistan, with a female specimen recorded from western China that I here tentatively assign to this species (Lackner 2010; Mazur 2011); *R. pavlovskii* is known currently only from eastern Turkmenistan, *R. sphingis* has been collected in southern Jordan and northern Egypt. Two newly described species, *R. aldhaferi* sp. n. and *R. perses* sp. n., are known only from the environs of Riyadh, Saudi Arabia and environs of Kerman, south-western Iran, respectively (Fig. 72).

***Reichardtiolus duriculus* (Reitter, 1904)**

http://species-id.net/wiki/Reichardtiolus_duriculus
Figs 1, 2, 4, 6, 8, 10, 12, 14–23

Saprinus duriculus Reitter, 1904: 31.
Styphrus duriculus: Jakobson (1911): 651.

Hypocacculus duriculus: Bickhardt (1916): 97.
Exaesiopus duriculus: Reichardt (1926): 17; Reichardt (1941): 330, 333, Fig. 172.
Reichardtiolus duriculus: Kryzhanovskij and Reichardt (1976): 239, Figs 465, 466, 468; Mazur (1984): 103; Mazur (1997): 265; Mazur (2004): 96; Lackner (2010): 187, Figs 27, 67, 132, 593–610; Mazur (2011): 210.

Type locality. Turkmenistan, Mary.

Type material examined. Holotype: ♀, side-mounted on a triangular point, four segments of meso-tarsomere broken off, last two meta-tarsomeres broken off, with the following labels: "♀" [printed]; followed by: "Merw" [printed]; followed by: "Ahnger" [printed]; followed by: "*S. duriculus* / m. 1904 Typ" [written label]; followed by: "coll. Reitter" [printed]; followed by: "1960 / *Exaesiopus* / (*Reichardtiolus*) / *duriculus* Rchdt (sic!) / Kryzhanovskij det." [printed-written]; followed by: "Holotypus 1904 / *Saprinus* / *duriculus* / Reitter" [red-framed printed-written label] (HNHM).

Additional material examined. TURKMENISTAN: 1 ♂, Anau, Karakum, 21.iv.1981, A. Olexa lgt.; 1 ♀ & 1 spec., Repetek, 12.iv.1989, M. Nikodým lgt.; 1 ♀, Amurdarja-Kirki, 1.-5.v.1993, no collector (all exs. TLAN); 1 spec., Karakum, Repetek, 4.v.1983, Krivoshatsky lgt., at light; 1 spec., Tschardshou, Repetek, 14.iv.1983, Snížek lgt. (both CPV); 4 specs., ibid, but MSNG; 1 spec., Repetek, in burrow of *Rhombomys opimus*, 1.iv.1980, Krivoshatskij lgt. (ZIN); 1 spec., ibid, but 19.iv.1982, at light, same collector (ZIN); 1 spec., 20 km E of Kerka, 23.iv.1984, at light, T. Vereschagina lgt. (ZIN). **KAZAKHSTAN**: 1 ♀, Temir env., river Chatryly, 26.v.1908, D. Borodin & B. Uvarov lgt. (ZIN); 2 specs., Mangyshlak peninsula, Schtepe env., 24.-27.iv.1999, Smirnov leg (CAS); 1 spec., without further data (MSNG); 1 spec., low Ili River, env. Bakanas, 15.iv.1971, Badenko lgt. (ZIN); 1 spec., Gurivskaya oblast, Makata distr., prom. Iskair, 13.vi.1981, Saraev lgt. (ZIN). **UZBEKISTAN**: 1 ♀, Syr-Darya gebiet, Perovsk uezd, 5.v.1905, J. Baeckmann lgt. (ZIN); 1 ♀, Kyzyl-Kum, Yny-Darja, Perovsk uezd, 24.iv.1911, Ivanov lgt. (ZIN); 2 specs., Kyzyl-Kum, Ayak-Agytma, 20.iv.1965, G. Medvedev lgt., sands (ZIN); 1 spec., Kyzyl-Kum, 70 km S of Tamdy, 1.v.1965, L. Arnoldi lgt. (ZIN). **TAJIKISTAN**: 1 ♀, Syr-Daria Riv., nr. Karakum Reservoir, at 40°32'16"N 70°17'47"E, 13.iv.61, sandy desert, I.K.Lopatin lgt. (CAT). **CHINA**: 1 ♀, Xinjiang Prov., mountain range Tokuz-Daban, upper Cherchen [=Qarqan] River, v. [18]90, Pevtzov lgt. (with doubt) (ZIN).

Re-description. Although this species has been recently re-described by the author (Lackner 2010: 187), and the reader is referred there for the exhaustive account of SEM micrographs and drawings of the mouthparts and sensory structures of the antenna, I prefer to repeat its re-description here for the reason that the following three species (*R. sphingis*, *R. aldhaferi* and *R. perses*) are morphologically very similar to *R. duriculus*. Those species are consequently provided only with diagnostic descriptions illuminating their respective differences from *R. duriculus*.

Body length: PEL: 2.00–3.40 mm; APW: 0.65–1.05 mm; PPW: 1.375–2.40 mm; EL: 1.25–2.25 mm; EW: 1.50–2.70 mm. Body (Fig. 1) elongate oval, strongly convex, cuticle dark brown with feeble metallic luster; legs, antennae and mouthparts rufous.

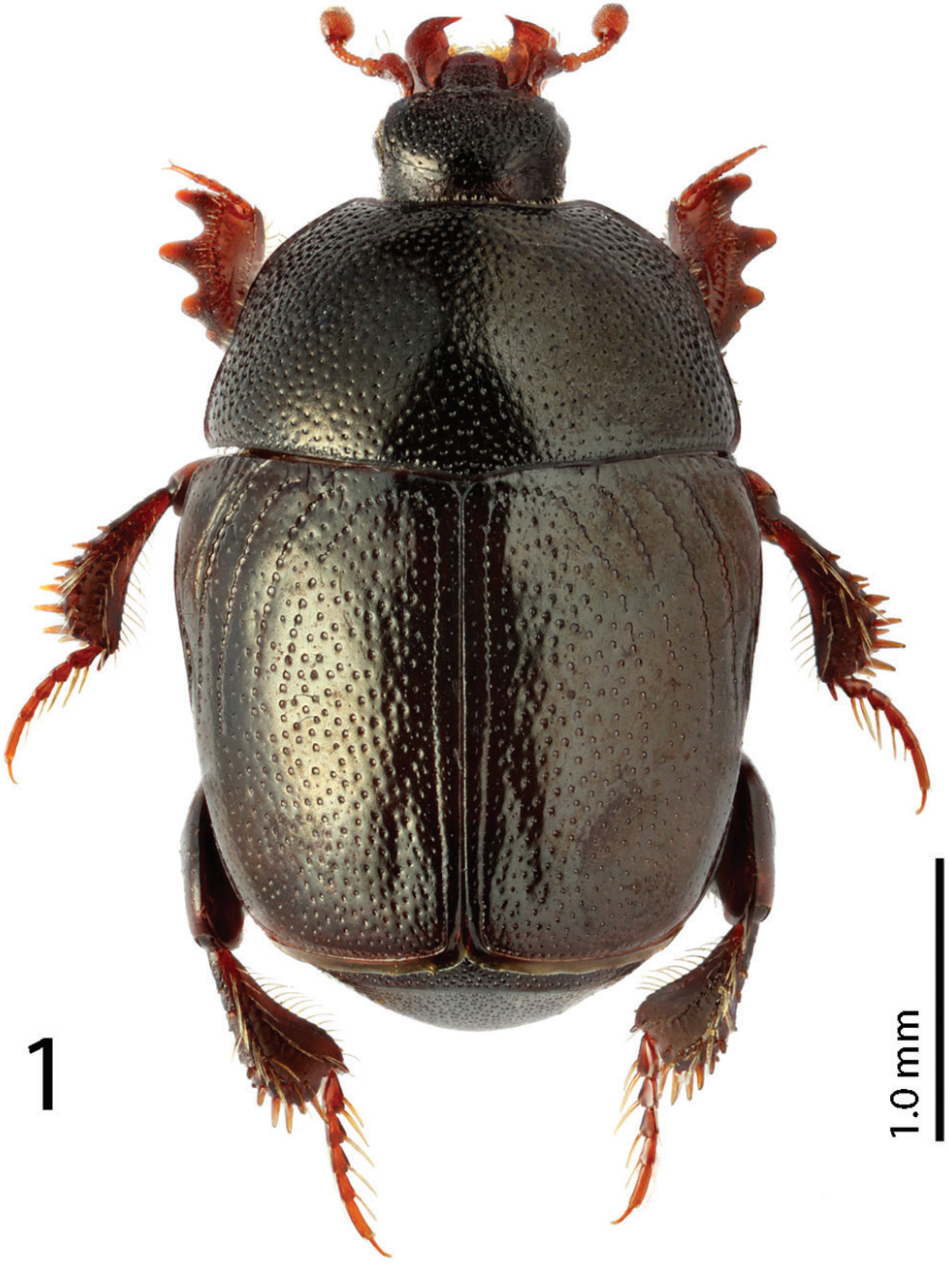


Figure 1. *Reichardtiolus duriculus* (Reitter, 1904) habitus. (Photo by M. Smirnov, Ivanovo, Russia).

Antennal scape (for fig. see Lackner 2010, fig. 596) slightly thickened, with several short setae; club (for fig. see Lackner 2010, fig. 595) rather large, without visible articulation, apical four-fifths covered with short sensilla intermingled with longer sparse

erect sensilla, basal fifth glabrous; sensory structures of antennal club (for fig. see Lackner 2010, fig. 27) in form of stipe-shaped vesicle situated under circular sensory area on internal distal margin of the ventral side of antennal club.

Mouthparts: mandibles (for fig. see Lackner 2010, fig. 101) with rounded outer margin, strongly curved inwardly, mandibular apex acutely pointed; sub-apical tooth on inner margin of left mandible blunt; labrum (for fig. see Lackner 2010, fig. 67) convex, coarsely punctate; with two labral pits, each with two well-sclerotized setae; terminal labial palpomere thickened, its width about half its length; mentum (Fig. 4) sub-trapezoidal, anterior margin shallowly emarginate medially; antero-lateral corners with few short setae, lateral margins with a single row of short ramose setae; disc of mentum imbricate, asetose; cardo of maxilla with few short setae on lateral margin; stipes triangular, with three short setae; terminal maxillary palpomere thickened, its width about half its length, about twice as long as penultimate.

Clypeus (Fig. 2) slightly concave medially, rounded laterally, rugulose-lacunose; frontal stria well impressed, carinate, almost straight, somewhat weakened medially, continued as well-impressed, carinate supraorbital stria; frontal disc (Fig. 2) densely punctate; eyes slightly convex, visible from above.

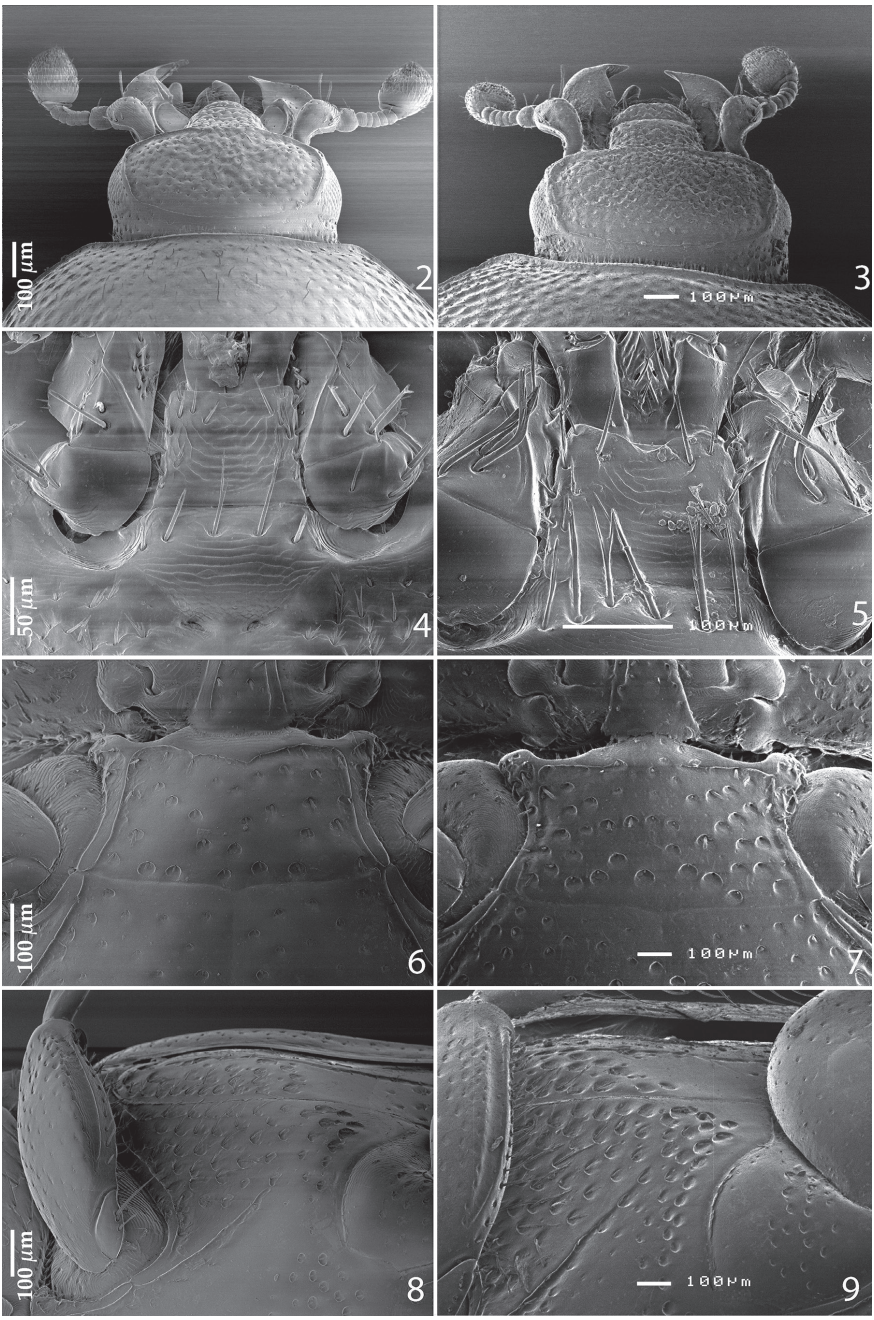
Pronotum (Fig. 1) convex, pronotal sides rounded, convergent anteriorly on their apical third, apical angles inconspicuous; marginal pronotal stria complete, carinate; disc with very deep, dense and coarse punctures, laterally rugulose-lacunose, medially punctuation weakens and becomes sparser; pronotal hypomeron with sparse short amber setae.

Elytral epipleuron with a row of deep punctures; marginal epipleural stria well impressed, complete; marginal elytral stria complete, deeply impressed, carinate, continued as complete apical elytra stria. Humeral elytral stria weakly impressed on basal third, often doubled; inner subhumeral stria inconspicuous, present as tiny median fragment; elytra with four dorsal striae 1–4, in large punctures, first, second and third dorsal striae about the same length, reaching approximately elytral half apically, fourth dorsal elytral stria weakly impressed on basal third (occasionally longer apically), connected to complete sutural elytral stria. Elytral disc with deep round punctuation, punctures separated by 2–4 times their diameter, becoming finer apically and laterally; between sutural elytral stria and elytral suture a row of regular fine punctures present.

Propygidium transverse, coarsely and densely punctate; pygidium (Fig. 12) almost as long as broad, with sparser punctuation; interspaces in both cases finely imbricate.

Anterior margin of median portion of prosternum (Fig. 10) rounded; marginal prosternal stria present laterally and as vague anterior fragment; prosternal foveae rather small; prosternal process rather narrow, slightly concave; carinal prosternal striae slightly carinate, almost parallel, united in front of strongly carinate, shortened lateral prosternal striae. Surface between carinal prosternal striae almost smooth, prosternal apophysis with several microscopic setae; lateral parts of prosternal process strigulate with scattered microscopic punctures fringed with tiny setae.

Anterior margin of mesoventrite (Fig. 6) feebly emarginate medially; discal marginal mesoventral stria well-impressed, carinate, slightly weakened anteriorly; disc of



Figures 2–9. 2 *Reichardtiolus duriculus* (Reitter, 1904) head, dorsal view **3** *Reichardtiolus sphingis* (Peyerimhoff, 1936), comb. n., head, dorsal view **4** *Reichardtiolus duriculus* (Reitter, 1904) mentum, ventral view **5** *Reichardtiolus sphingis* (Peyerimhoff, 1936), comb. n., mentum, ventral view **6** *Reichardtiolus duriculus* (Reitter, 1904) mesoventrite **7** *Reichardtiolus sphingis* (Peyerimhoff, 1936), comb. n., mesoventrite **8** *Reichardtiolus duriculus* (Reitter, 1904) lateral disk of metaventrite **9** *Reichardtiolus sphingis* (Peyerimhoff, 1936), comb. n., lateral disk of metaventrite.

mesoventrite with scattered deep, round punctures, fringed with microscopic setae; meso-metaventral sutural stria absent; meso-metaventral suture distinct.

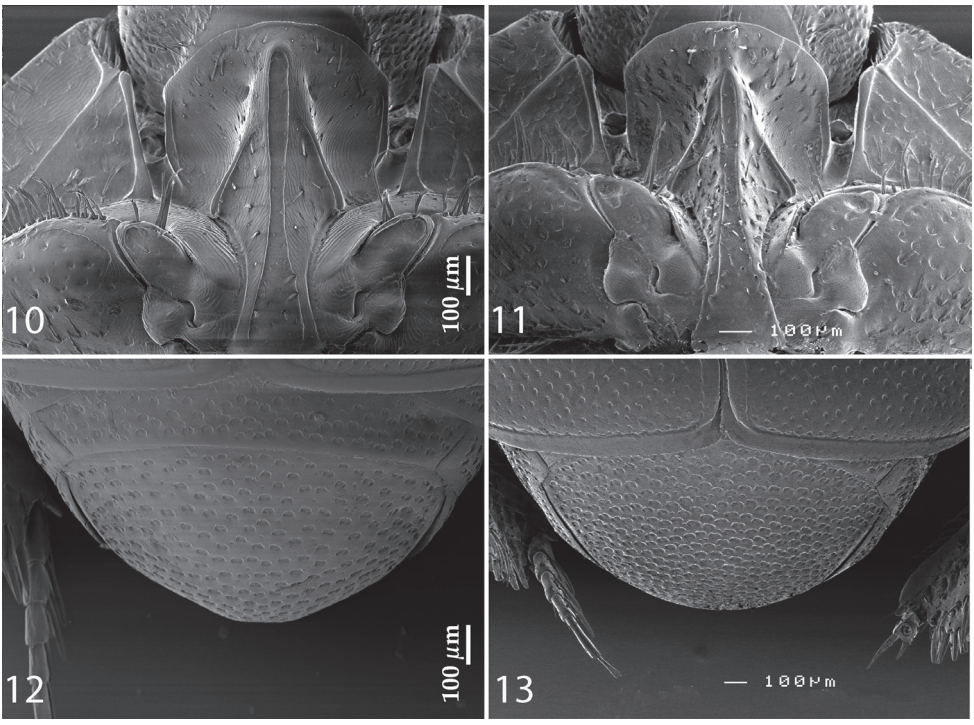
Intercoxal disc of metaventrite slightly longitudinally concave in male, with coarse scattered punctures, area around lateral metaventral stria smooth; lateral metaventral stria (Fig. 8) deeply impressed, carinate, extending obliquely and shortened apically; lateral disc of metaventrite (Fig. 8) with shallow large setiferous punctures; metepisternum on basal half with similar punctuation, apical half of metepisternum (Fig. 8) almost smooth, fused metepimeron with few punctures; metepisternal stria present along entire fused metepimeron and metepisternum, intermittent basally.

Intercoxal disc of first abdominal sternite completely striate laterally, with sparse coarse punctuation.

Protibia (for fig. see Lackner 2010, fig. 603) flattened and somewhat dilated, apical protibial margin formed by anterior margin of large sub-triangular distal-most tooth topped with large triangular denticle, outer margin apart from this tooth with another similar tooth topped with large triangular denticle, followed by another, much lower tooth topped by much smaller triangular denticle and another microscopic denticle entombed in outer margin of protibia; setae of outer row on anterior surface of protibia sparse, regular and short; setae of intermediate row similarly sparse and regular, much shorter than those of outer row; protarsal groove moderately deep; anterior protibial stria present only on basal third; tarsal denticles absent; protibial spur tiny, bent, growing out from apical protibial margin; apical margin of protibia posteriorly without denticles; outer part of posterior surface of protibia sparsely punctate, distinctly separated from glabrous median part of posterior surface by irregular costiform stria fringed with sparse microscopic setae; posterior protibial stria complete, deeply impressed, with sparse microscopic setae; inner-ventral denticles absent; inner margin with single row of well sclerotized setae.

Mesotibia (for fig. see Lackner 2010, fig. 601) slightly thickened, outer margin with two sparse rows of thin denticles greater in size apically; setae of outer row rather dense, strongly sclerotized and longer than denticles of outer margin; setae of intermediate row sparse, microscopic; posterior mesotibial stria inconspicuous; anterior surface of mesotibia imbricate, with scattered minuscule punctures with microscopic setae; anterior mesotibial stria shortened apically, almost complete; mesotibial spur stout, rather short; apical margin with several tiny denticles; claws of apical tarsomere longer than half its length; metatibia basically similar to mesotibia, but much more thickened and dilated, rows of denticles of outer margin widely separated, outer row of denticles (for fig. see Lackner 2010, fig. 602) observable only from ventral view.

Male genitalia: Eighth sternite (Figs 14–15) divided medially, apically with short setae and a setose velum, 8th tergite apically only faintly emarginate, 8th sternite and tergite fused laterally, deep from lateral view (Fig. 16). Tenth tergite (Fig. 17) basally almost straight; 9th tergite apically inwardly arcuate, anterior angles prominent (Fig. 17), sclerotization not divided medially. Spiculum gastrale (Figs 19–20): tips on anterior end without strong sclerotization, posterior end outwardly arcuate. Basal piece of aedeagus (Figs 22–23) rather short, ratio to tegmen 1:5; aedeagus tube-like, with large opening for median lobe, apically with numerous pseudopores, curved laterally (Fig. 22); apex of aedeagus blunt (Fig. 21).



Figures 10–13. 10 *Reichardtiolus duriculus* (Reitter, 1904) prosternum **11** *Reichardtiolus sphingis* (Peyerimhoff, 1936), comb. n., prosternum **12** *Reichardtiolus duriculus* (Reitter, 1904) pygidium **13** *Reichardtiolus sphingis* (Peyerimhoff, 1936), comb. n., pygidium.

Differential diagnosis. *R. duriculus* is most readily separated from *R. pavlovskii* from which it differs by the body size and other substantial morphological characters, e.g. the presence (vs. absence) of prosternal foveae, presence of elytral striae (almost indiscernible in *R. pavlovskii*) etc. The differences among *R. duriculus* and other three congeners are subtler and the species are best separated by their male terminalia; the reader is referred to the key to species for details.

Biology. A psammophilous species, usually collected in sand, occasionally collected also in rodent's burrows or even at light.

Distribution. Turkmenistan, Kazakhstan, Uzbekistan, western China (?). Newly recorded from Tajikistan (Fig. 72).

Remarks. The single specimen from Xinjiang is a female, and differs from the specimens from ex-Soviet middle Asia especially by very coarsely and rugosely punctate frons and clypeus, as well as denser and coarser punctuation of mesoventrite and pygidium. However, I am hesitant to describe a new species based on a single female and prefer rather keeping it tentatively as a specimen of *R. duriculus*. Certainly, acquisition of new material containing male specimens from the above-mentioned locality would help clarify its taxonomic status.

***Reichardtiolus sphingis* Peyerimhoff, 1936, comb. n.**
http://species-id.net/wiki/Reichardtiolus_sphingis
Figs 3, 5, 7, 9, 11, 13, 24–33

Saprinus sphingis Peyerimhoff, 1936: 221; Mazur 1984: 64; Mazur 1997: 232; Mazur 2004: 101; Mazur 2011: 188.

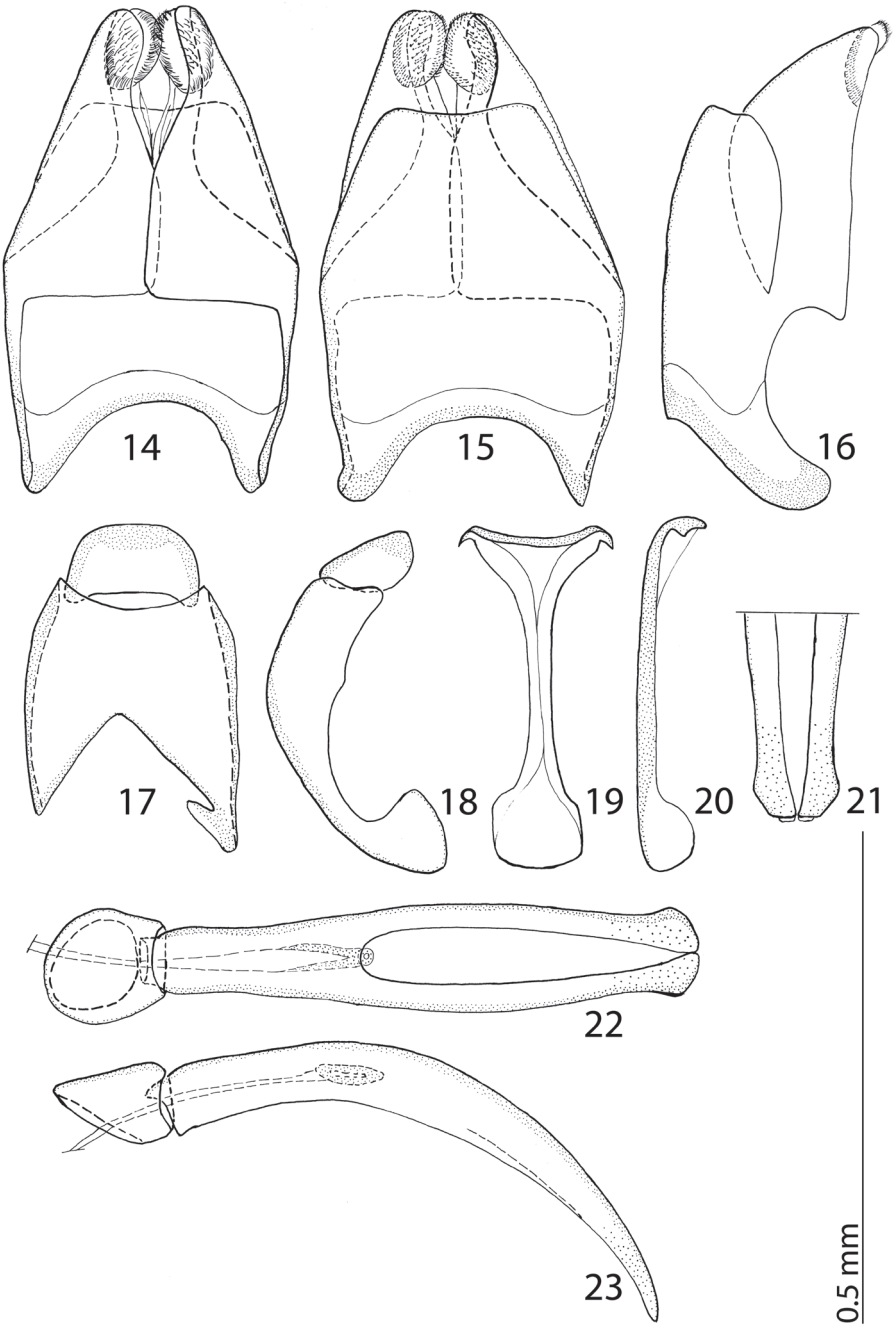
Type locality. Egypt, Sakkara.

Material examined. EGYPT: 1 ♀, Gebel Asfar, 2.iv.1935, coll. Alfieri Egypt (FMNH). **JORDAN**: 2 ♂♂, 1 ♀ & 9 specs., 60 km N El Mudawwara, 1000 m, 29°20'N, 35°32'E, 5.iv.1994, Bečvář J. & S. lgt. (TLAN); 1 ♂, ibid, but CAT; 1 ♂, ibid, but CND; 10 ♀♀, ibid, but MSNG, 1 ♂ & 1♀, ibid, but CYG.

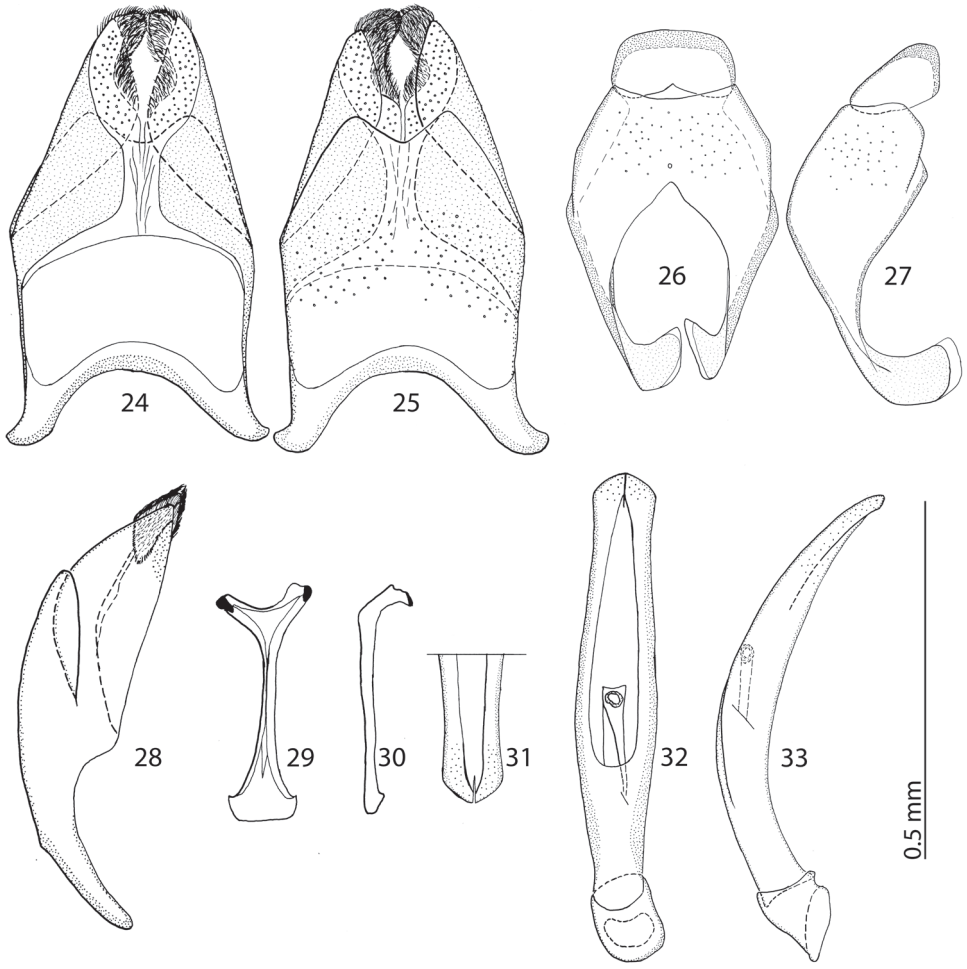
Diagnostic description. Body size: PEL: 2.80–3.25mm; APW: 0.90–1.10mm; PPW: 2.00–2.40mm; EW: 2.25–2.65mm; EL: 1.75–2.10mm. Body as in *R. duriculus*, pronotum darker than elytra; legs, antennae and mouthparts rufous; antennae as in *R. duriculus*. Mouthparts as in *R. duriculus*, but mentum on its anterior margin with deeper emargination (compare Figs 4 and 5). Clypeus and frons similar to *R. duriculus* (compare Figs 2 and 3), but punctuation coarser and denser. Structure of pronotum and elytra similar to those of *R. duriculus*; punctuation of elytral disk somewhat sparser than that of *R. duriculus*. Propygydium and pygydium more coarsely punctate than those of *R. duriculus*, otherwise similar to it (compare Figs 12 and 13). Prosternum similar to that of *R. duriculus*, but more densely punctate (compare Figs 10 and 11). Mesoventrite similar to that of *R. duriculus*, but marginal mesoventral stria of *R. sphingis* anteriorly interrupted medially and rather straight (compare Figs 6 and 7). Metaventrite similar to that of *R. duriculus*, but lateral disk of metaventrite and metepisternum more coarsely punctate than those of *R. duriculus* (compare Figs 8 and 9). Abdominal ventrites similar to those of *R. duriculus*. Legs similar to those of *R. duriculus*, but teeth of protibia of *R. sphingis* more blunt than those of *R. duriculus* and denticles of meso- and metatibia of *R. sphingis* shorter, thinner and more blunt than those of *R. duriculus*. Male genitalia: 8th sternite (Figs 24–25) well sclerotized, apically with small setose velum covered with pores; 8th tergite (Fig. 25) apically widely emarginated medially, covered with pores and pseudopores. 9th tergite (Fig. 26) strongly sclerotized laterally, anterior half with pores and pseudopores, laterally with projection (Fig. 27); basal margin of 10th tergite inwardly arcuate (Fig. 26). Spiculum gastrale (Fig. 29) on anterior end strongly sclerotized on both tips; posterior end almost straight. Aedeagus of *R. sphingis* similar to that of *R. perses* (compare Figs 32–33 and 60–61); aedeagal apex of *R. perses* blunt, whereas pointed in *R. sphingis* (compare Figs 31 and 58).

Differential diagnosis. *R. sphingis* is best separated from *R. pavlovskii* by the same characters as *R. duriculus*; for the differences among rest of the congeners the reader is referred to the key to species.

Biology. According to Mr. S. Bečvář (pers. comm.) the series of this species from Jordan (El Mudawwara) was found under the grass at the foot of a small sand dune.



Figures 14–23. 14 *Reichardtiolus duriculus* (Reitter, 1904) 8th sternite and tergite, ventral view **15** ditto, dorsal view **16** ditto, lateral view **17** *Reichardtiolus duriculus* (Reitter, 1904) 9th + 10th tergites, dorsal view **18** ditto, lateral view **19** *Reichardtiolus duriculus* (Reitter, 1904) spiculum gastrale, ventral view **20** ditto, lateral view **21** *Reichardtiolus duriculus* (Reitter, 1904) apex of aedeagus, frontal view **22** *Reichardtiolus duriculus* (Reitter, 1904) aedeagus, dorsal view **23** ditto, lateral view.



Figures 24–33. 24 *Reichardtiolus sphingis* (Peyerimhoff, 1936), comb. n., 8th sternite and tergite, ventral view **25** ditto, dorsal view **26** *Reichardtiolus sphingis* (Peyerimhoff, 1936), comb. n., 9th + 10th tergites, dorsal view **27** ditto, lateral view **28** *Reichardtiolus sphingis* (Peyerimhoff, 1936), comb. n., 8th sternite and tergite, lateral view **29** *Reichardtiolus sphingis* (Peyerimhoff, 1936), comb. n., spiculum gastrale, ventral view **30** ditto, lateral view **31** *Reichardtiolus sphingis* (Peyerimhoff, 1936), comb. n., apex of aedeagus, frontal view **32** *Reichardtiolus sphingis* (Peyerimhoff, 1936), comb. n., aedeagus, dorsal view 33 ditto, lateral view.

Distribution. Egypt, surroundings of Cairo; south Jordan, 60 km N El Mudawwara (Fig. 72).

Remarks. Peyerimhoff (1936) based his description of *Saprinus sphingis* on a single female, collected on 12 January 1933 in Sakkara, which is in northern Egypt (Peyerimhoff's original description mentions "Basse-Egypte"), vicinity of Cairo. The type specimen was, according to Peyerimhoff's description deposited in Alfieri's collection. Although this collection has been (partly?) acquired by FMNH, the only specimen of *S. sphingis* found there did not bear the locality labels corresponding with those of

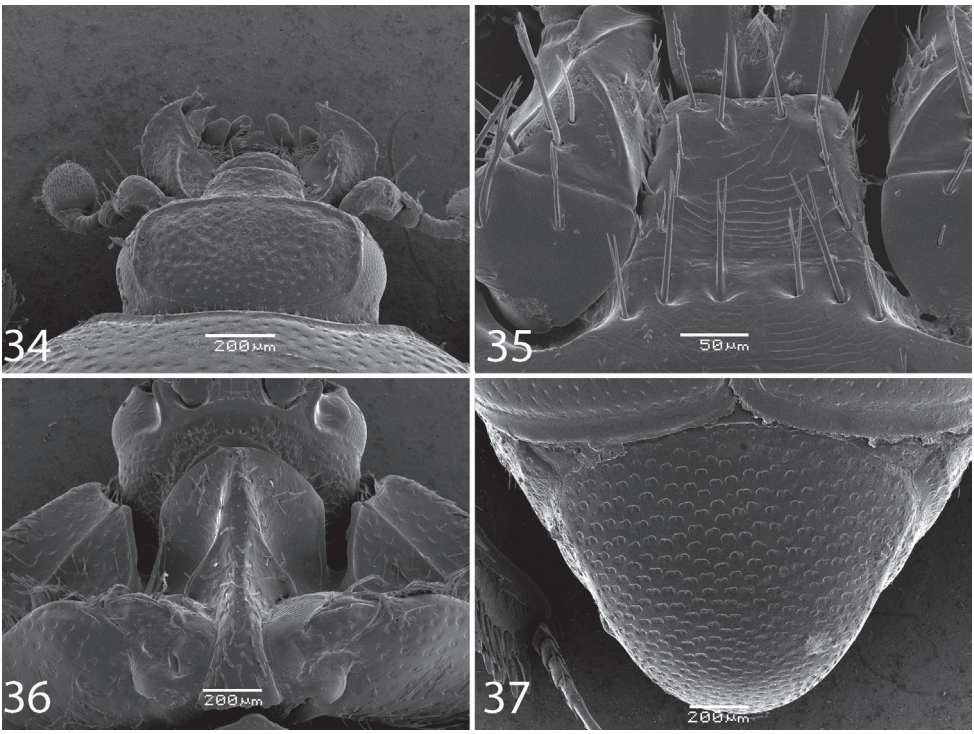
the Peyerimhoff's type specimen. Therefore this specimen cannot be designated as the Lectotype and the type specimen of *Saprinus sphingis* remains undiscovered. However, the specimen treated here was most likely identified by Peyerimhoff as *S. sphingis* and completely agrees with Peyerimhoff's description. It has been collected near Jebel Asfar, which is north of Cairo. This locality is not far from Sakkara, which is south of Cairo. The specimens collected in southern Jordan by Mrs. J. & S. Bečvář (České Budějovice, Czech Republic) are virtually identical to the specimen from Egypt. Because the only known specimen of *R. sphingis* from Egypt is a female, the genitalia depicted in this work belong to one of the Jordanian specimens.

***Reichardtiolus aldhaferi* sp. n.**
http://zoobank.org/5DBC0C28-18FC-40FA-92B4-21222C33DE98
http://species-id.net/wiki/Reichardtiolus_aldhaferi
Figs 34–47

Type locality. Saudi Arabia, environs of Riyadh, Rhodet Khorim.

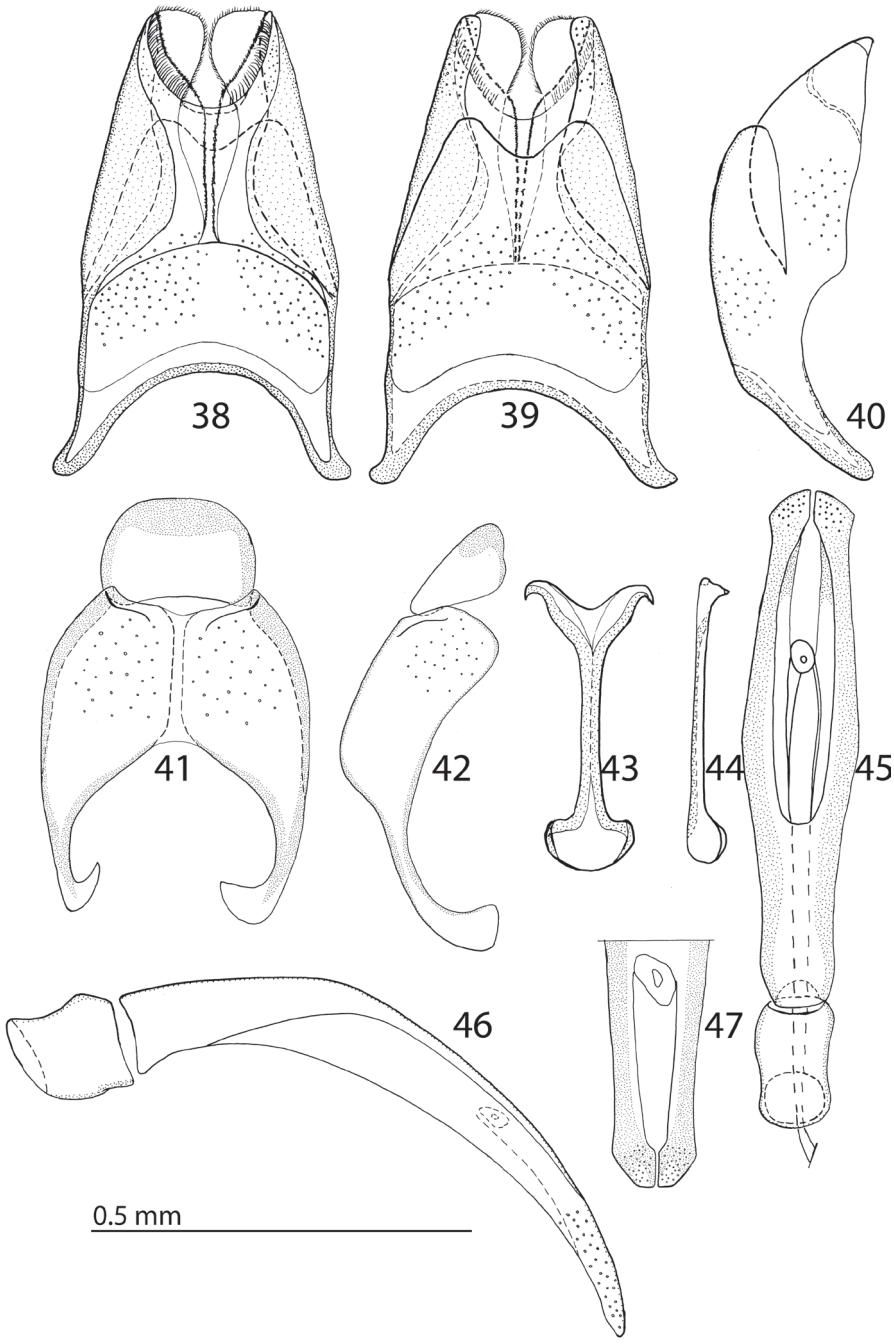
Type material examined. Holotype, male, side-mounted on a triangular point with male genitalia extracted, dismembered and glued to the same mounting-point as the specimen, with following labels: "♂" (printed); followed by: "Saudi Arabia, Rhodet Khorim / 25°25.943'N, 47°13.863', Alt. / 572m 5.ii.2012 HP (B)" (printed, black-margined label); followed by: "*Reichardtiolus aldhaferi* / sp. n. Det. T. Lackner / 2013 HOLOTYPE" (red label, printed) (KSMA). Paratypes: 3 ♂♂ & 1 ♀, idem as Holotype (1 ♂ and 1 ♀ are sputter-coated with gold); 2 ♀♀, with following labels: "♀" (printed), followed by: "Saudi Arabia, Rhodet Khorim / N : 25°22'58"/E:47°16'44" / 08.i.2012 Light Trap (A) (printed, black-margined label); 1 ♀, with following labels: "♀" (printed), followed by: "Saudi Arabia Rhodet Khorim / N : 25°25'94"/ E: 47°13'86" / 25.xii.2011 Light Trap (B) (printed, black-margined label); 1 ♀, with following labels: "♀" (printed), followed by: "Saudi Arabia Kharah, Al / Mozahmiah 30km W.Riyadh / 24.ii.2011/LT / N28°23'33"/ E46°14'39" / Al Dhafer, H.; Kondratieff,B.; / Fadl, H.&Al Gharbawi, A. (printed-written, black-margined label); 1 ♀, with following labels: "♀" (printed), followed by: KSA: Riyadh: Dirab / 20.i.1986 LT (written). All exs. KSMA except for 1 ♂ from Rhodet Khorim, 5.ii.2012 and 1 ♀, ibid, but 25.xii.2011 in coll. TLAN.

Diagnostic description. Body size: PEL: 2.50–3.25mm; APW: 0.85–1.15 mm; PPW: 1.80–2.25 mm; EW: 2.00–2.50 mm; EL: 1.50–2.00 mm. Body darker than that of *R. duriculus*, otherwise similar to it. Legs and antennae darker than those of *R. duriculus*; mouthparts similar except mentum, which is on its anterior margin more emarginated than that of *R. duriculus* (compare Figs 4 and 35). Clypeus anteriorly elevated (Fig. 34), with slight median depression, rugosely punctate; frons (Fig. 34) coarsely and densely punctate, medially rugulose-lacunose, with shallow depressions; frontal and supraorbital striae and eyes as in *R. duriculus*. Pronotum slightly less acutely narrowing apically than that of *R. duriculus*; punctuation on pronotal disk sparser than that of *R. duriculus*. Elytra similar to those of *R. duriculus*, but dorsal elytral striae weaker, occasionally striae 3-4



Figures 34–37. 34 *Reichardtiolus aldhaferi* sp. n., head, dorsal view **35** *Reichardtiolus aldhaferi* sp. n., mentum, ventral view **36** *Reichardtiolus aldhaferi* sp. n., prosternum **37** *Reichardtiolus aldhaferi* sp. n., pygidium.

shortened apically, only half as long as striae 1-2 or even evanescent; between 4th dorsal elytral and sutural striae in several specimens punctures scratch-like and surface with variously deep longitudinal wrinkles; rarely with shallow depression between the bases of 4th and sutural elytral striae. Punctuation of elytral disk sparser than that of *R. duriculus*, punctures separated by several times their diameter; in fourth elytral interval occasionally scratch-like. Propygidium and pygidium similar to those of *R. duriculus*, but punctuation denser and coarser in *R. aldhaferi*, although not as dense as in *R. sphingis* (compare Figs 12, 13 and 37). Structure of prosternal process similar to that of *R. duriculus*, but prosternal keel laterally more compressed and setose (compare Figs 10 and 36); carinal prosternal striae occasionally very approximate, medially almost united and difficult to discern; prosternal foveae smaller than those of *R. duriculus*. Mesoventrite sub-square, trapezoidal, punctuation sparse, punctures separated by several times their own diameter; marginal mesoventral stria always complete anteriorly, almost straight; meso-metaventral sutural stria absent, suture distinct. Metaventrite, metepisternum and abdominal ventrites similar to those of *R. duriculus*. Legs as in *R. duriculus*; except denticles of mesotibia that are sparser, thinner and shorter. Male genitalia: 8th sternite (Figs 38–39) strongly sclerotized laterally, apically with pseudopores and a row of short setae and small velum covered with minute setae; 8th tergite (Fig. 39) deeply emarginated apically, on basal half



Figures 38–47. 38 *Reichardtiolus aldhaferi* sp. n., 8th sternite and tergite, ventral view **39** ditto, ventral view **40** ditto, lateral view **41** *Reichardtiolus aldhaferi* sp. n., 9th + 10th tergites, dorsal view **42** ditto, lateral view **43** *Reichardtiolus aldhaferi* sp. n., spiculum gastrale, ventral view **44** ditto, lateral view **45** *Reichardtiolus aldhaferi* sp. n., aedeagus, dorsal view **46** ditto, lateral view **47** *Reichardtiolus aldhaferi* sp. n., apex of aedeagus, frontal view.

with prominent pores; 8th sternite and tergite fused laterally (Fig. 40). 9th tergite (Fig. 41) well sclerotized along margins, laterally without projection (Fig. 42), apically with two bisinuate strongly sclerotized lines visible from dorsal view, apical half covered with pseudopores, sclerotization of tergite medially divided, two parts held together by weakly sclerotized part; 10th tergite basally faintly inwardly arcuate (Fig. 41). Tips of apical end of spiculum gastrale (Fig. 43) without strongly sclerotized parts, apical end strongly inwardly arcuate, basal end outwardly arcuate. Aedeagus (Figs 45–46) similar to that of *R. duriculus*, but laterally more curved and medially thickened (compare Figs 23 and 46).

Differential diagnosis. As with preceding species.

Biology. Unknown, presumably similar to the congeners, the examined specimens were collected at light in winter months.

Distribution. Saudi Arabia, environs of Riyadh (Fig. 72).

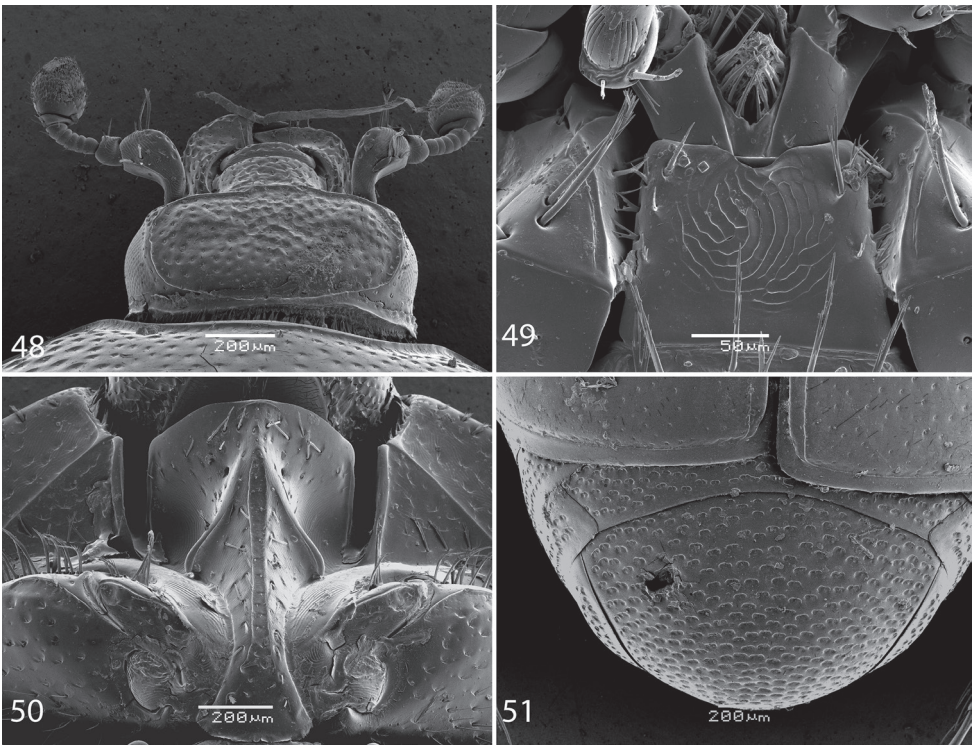
Etymology. Patronymic, named after the head of the entomology department at KSMA, H. M. Al Dhafer.

***Reichardtiolus perses* sp. n.**
http://zoobank.org/9B800BDD-A4B9-4D0B-BCE1-85CE9859E039
http://species-id.net/wiki/Reichardtiolus_perses
Figs 48–61

Type locality. Iran, Kerman, Talab.

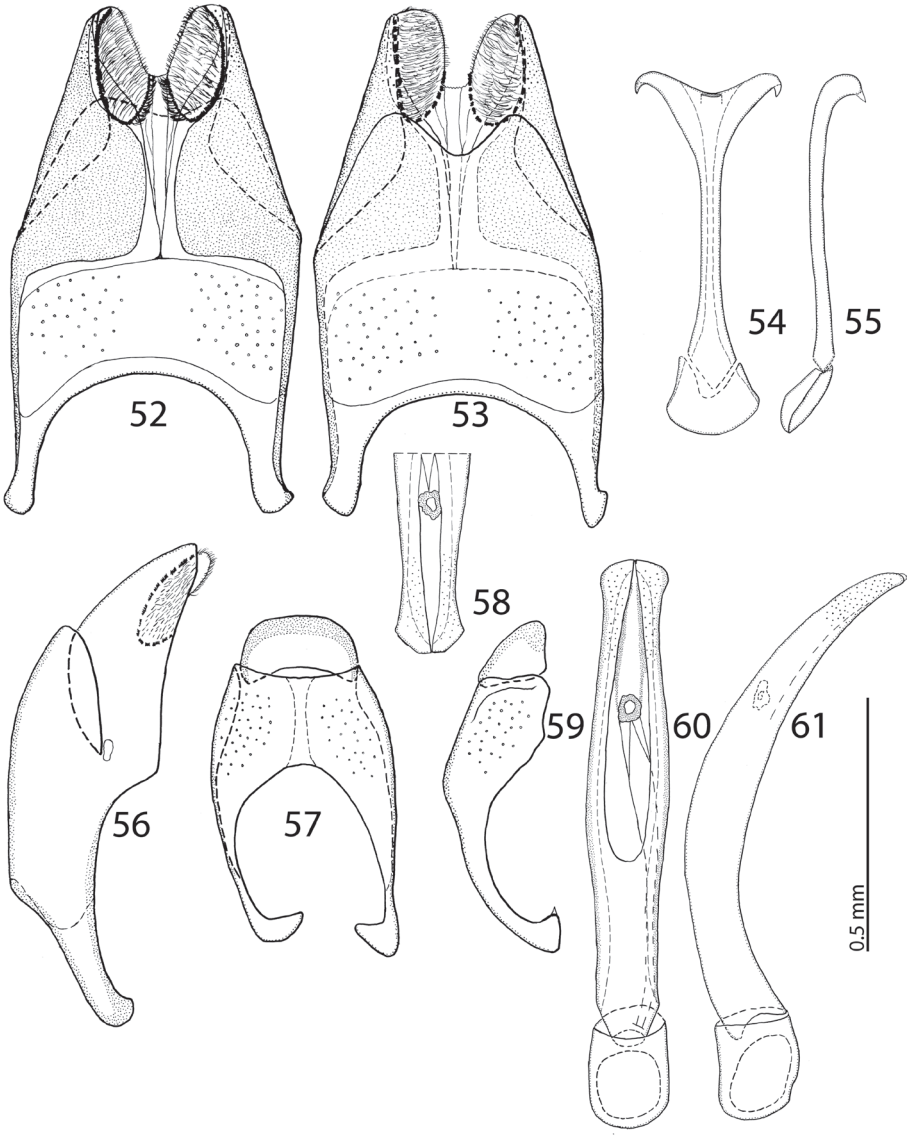
Type material examined. Holotype, male, side-mounted on triangular point with male genitalia extracted and glued to the same triangular point as the specimen, left protarsus and left mid-leg missing, piece of left elytron from the elytral flank along the elytral base towards the fourth elytral stria chipped out; with the following labels: "♂" (printed); followed by: "Kerman: str. Talab / 19–20.i.[19]01 / N. Zarudny" (printed-written label in Russian); followed by: "Coll. Semenov-Tian-Shansky" (printed); followed by: "ZOOLOGICAL / INSTITUTE RAS / ST. PETERSBURG" (yellow label, printed); followed by: "*Reichardtiolus perses* / sp.nov. HOLOTYPE / Det. T. Lackner 2013" (red label, printed) (ZIN). Paratypes: 1 ♀, ibid (sputter coated with gold) (ZIN); 1 ♀, ibid, but 20.i.[19]01, with an additional written-printed label: "*Exaesiopus* / *duriculus* Rtt. / Reichardt det." (TLAN).

Diagnostic description. Body size: PEL: 2.50–3.75 mm; APW: 0.75–1.15 mm; PPW: 1.90–2.75 mm; EW: 2.00–3.00 mm; EL: 1.75–2.50 mm. Body in general (except for *R. pavlovskii*) larger than the rest of congeners, cuticle similar to that of *R. duriculus*; legs, antennae and mouthparts chestnut brown. Mouthparts similar to those of *R. duriculus*, mentum on anterior margin deeply emarginated medially (Fig. 49). Clypeus and frons (Fig. 48) coarsely and densely punctate; frontal stria weakened medially; frontal disk with low protuberances and shallow depressions, very coarsely and densely punctate, especially medially; clypeus margined laterally. Pronotum as in *R. duriculus*, punctuation medially sparser, punctures weak and separated by several times their diameter. Elytra generally similar to those of *R. duriculus*;



Figures 48–51. 48 *Reichardtiolus perses* sp. n., head, dorsal view **49** *Reichardtiolus perses* sp. n., mentum, ventral view **50** *Reichardtiolus perses* sp. n., prosternum **51** *Reichardtiolus perses* sp. n., pygidium.

punctuation of pygydium generally denser than that of *R. duriculus* (compare Figs 12 and 51). Prosternal process flattened to slightly concave, compressed laterally; carinal prosternal striae approximate, complete; prosternal foveae small. Mesoventrite sub-quadrate, marginal stria anteriorly complete; punctuation sparser than that of *R. duriculus*, punctures separated by several times their diameter; meso-metaventral stria absent, in case of one specimen substituted by a string of punctures. Metaventrite, metepisternum and abdominal ventrites similar to those of *R. duriculus*. Legs similar to those of *R. duriculus*, *R. sphingis*, and *R. aldhaferi*. Male genitalia: 8th sternite (Figs 52–53) strongly sclerotized, apically with dense row of short setae and setose velum; 8th tergite apically with deep emargination, on basal half with numerous pores and pseudopores (Fig. 53). Sclerotization of 9th tergite divided medially (as in *R. aldhaferi*), on apical half with pores and pseudopores; 10th tergite inwardly arcuate on its basal margin. 9th tergite on apical third with faint, weakly sclerotized bisinuate line, visible only from lateral view (Fig. 59). Spiculum gastrale (Fig. 54) on apical end inwardly arcuate (although not as deeply as with *R. sphingis* or *R. aldhaferi*), with a unique sclerotized ring medially; basal end of spiculum gastrale outwardly arcuate. Aedeagus generally most similar to that of *R. sphingis*, but blunt apically (compare Figs 31 and 58).



Figures 52–61. 52 *Reichardtiolus perses* sp. n., 8th sternite and tergite, ventral view **53** ditto, dorsal view **54** *Reichardtiolus perses* sp. n., spiculum gastrale, ventral view **55** ditto, lateral view **56** *Reichardtiolus perses* sp. n., 8th sternite and tergite, lateral view **57** *Reichardtiolus perses* sp. n., 9th + 10th tergites, dorsal view **58** *Reichardtiolus perses* sp. n., apex of aedeagus, frontal view **59** *Reichardtiolus perses* sp. n., 9th + 10th tergites, lateral view **60** *Reichardtiolus perses* sp. n., aedeagus, dorsal view **61** ditto, lateral view.

Differential diagnosis. *R. perses* is the second largest species of the genus (after *R. pavlovskii*) and externally very similar to *R. duriculus*, *R. aldhaferi*, and *R. sphingis*, differing from them mainly by the structure of male terminalia. From the largest species of the genus, *R. pavlovskii* it differs by the same characteristics as the preceding three species.

Biology. Unknown, presumably similar to its congeners.

Distribution. Iran, environs of Kerman (Fig. 72).

Etymology. The name of this new species means "Persian". It is a noun in apposition in the nominative singular form.

Reichardtiolus pavlovskii Kryzhanovskij, 1959

http://species-id.net/wiki/Reichardtiolus_pavlovskii
Figs 62–71

Exaesiopus pavlovskii Kryzhanovskij, 1959: 216, fig 1.
Reichardtiolus pavlovskii: Kryzhanovskij in Kryzhanovskij and Reichardt (1976): 239, 240; Mazur (1984): 103; Mazur (1997): 265; Mazur (2004): 96; Mazur (2011): 210.

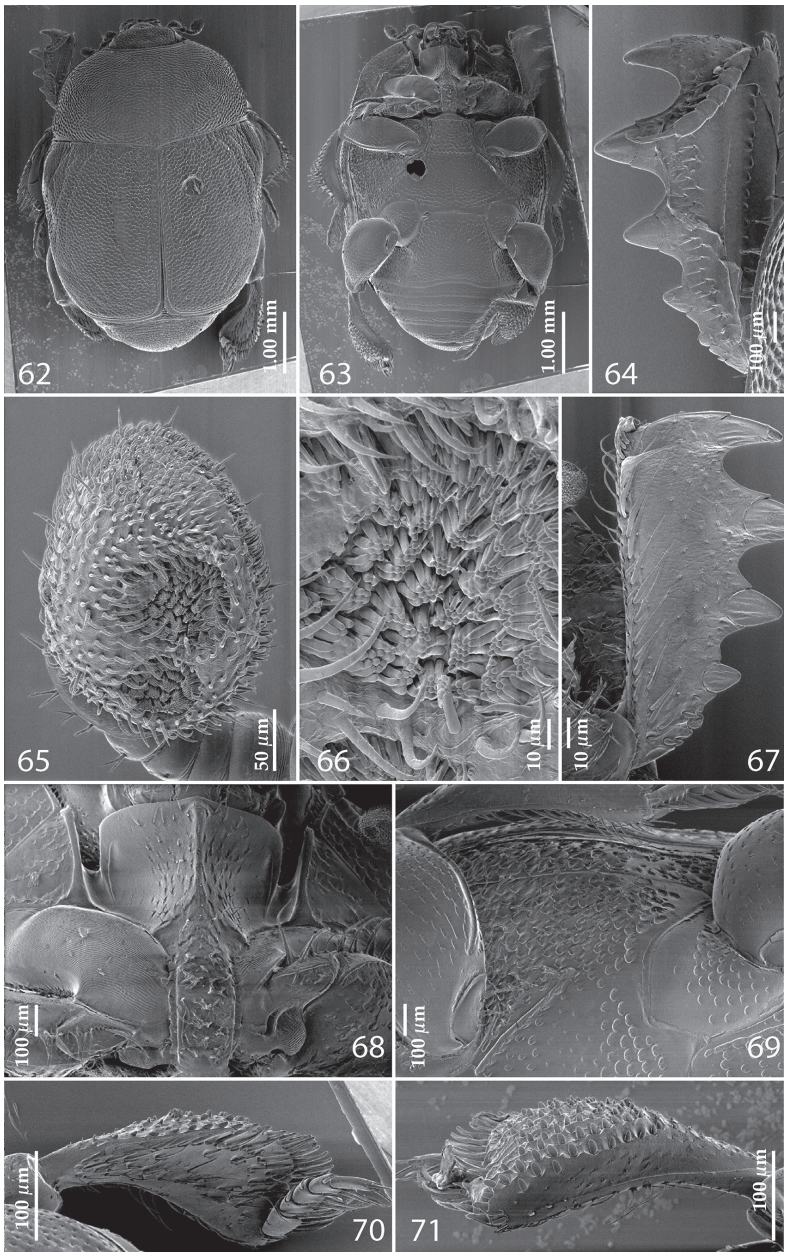
Type locality. Turkmenistan, Badkhyz Nature Reserve.

Type material examined. Holotype, female, side mounted on a triangular mounting point: "Yu. V. [=Yugo-Vostochnyj, South-Eastern] Turkm. [=Turkmenistan], Badkhyz / 12 km W Kala-i-Mor / 31.iii.1957 G. Medvedev" [written]; "Barkhannye peski [= moving sands]" (written); "*Exaesiopus* / (*Reichardtiolus*) / *pavlovskii* m., typ. / O. Kryzhano- / vskij det [1]958" (printed-written); "Holotypus / *Exaesiopus* / *pavlovskii* Kryzh." (red label, written); "Zoological / Institute RAS / St. Petersburg" (yellow printed label); "09-068" (yellow pencil-written label), added by the author (ZIN).

Re-description. Body size PEL: 4.25 mm; APW: 1.25 mm; PPW: 3.20 mm; EL: 3.50 mm; EW: 3.00 mm. Body (Figs 62–63) rectangular oval, strongly convex, pronotum somewhat narrower than elytra, cuticle dark brown to black, elytra somewhat lighter, without metallic luster, entire dorsal surface rugulose-lacunose; legs, mouthparts and antennae light to dark brown, antennal club black.

Antennal scape not particularly thickened, punctate dorsally, punctures with numerous long setae; club (Fig. 65) oval, slightly depressed dorso-ventrally; without visible articulation, entire surface with thick short yellow sensilla intermingled with sparse longer erect sensilla, ventrally with two large round sensory areas (Figs 65, 66); sensory structures of antennal club not examined. Mouthparts: mandibles stout, densely punctate, dorso-lateral area with sparse short setae, acutely pointed; labrum convex with two labral setae growing out from each labral pit; square-shaped, anterior angles produced, anterior margin with deep median excavation, surface around it with four longer setae; lateral margins with double row of shorter ramose setae; disc of mentum imbricate; other parts of the mouth not examined.

Clypeus sub-quadrate, coarsely punctate, slightly depressed medially and slightly carinate laterally; frontal stria carinate, interrupted anteriorly, continuous with weakly carinate supraorbital stria; frontal disc rugulose-lacunose; eyes flattened, but visible from above.



Figures 62–71. 62 *Reichardtiolus pavlovskii* (Kryzhanovskij, 1959) habitus, dorsal view **63** *Reichardtiolus pavlovskii* (Kryzhanovskij, 1959) habitus, ventral view **64** *Reichardtiolus pavlovskii* (Kryzhanovskij, 1959) protibia, dorsal view **65** *Reichardtiolus pavlovskii* (Kryzhanovskij, 1959) antennal club, ventro-lateral view **66** *Reichardtiolus pavlovskii* (Kryzhanovskij, 1959) detail of the sensory area of the antenna **67** *Reichardtiolus pavlovskii* (Kryzhanovskij, 1959) protibia, ventral view **68** *Reichardtiolus pavlovskii* (Kryzhanovskij, 1959) prosternum **69** *Reichardtiolus pavlovskii* (Kryzhanovskij, 1959) lateral disk of metaventrite + fused metepisternum **70** *Reichardtiolus pavlovskii* (Kryzhanovskij, 1959) metatibia, dorsal view **71** *Reichardtiolus pavlovskii* (Kryzhanovskij, 1959) ditto, ventral view.

Pronotal sides (Fig. 62) on basal two-thirds moderately convergent anteriorly, strongly convergent anteriorly on apical third, apical angles blunt; pronotal foveae absent; marginal pronotal stria complete, carinate, slightly weakened behind head; disc of pronotum completely with deep coarse elongate punctures separated by less than half their diameter forming rugulose-lacunose wrinkles medially; pronotal hypomeron with short yellow setae; scutellum very small, visible.

Elytral humeri slightly prominent; elytra widest at humeri; elytral epipleura in large punctures; marginal epipleural stria complete, surface between it and elytral margin smooth; marginal elytral stria straight and carinate, continued as somewhat weakened complete apical elytral stria continuous with sutural elytral stria. Humeral elytral stria faintly impressed on basal third; inner subhumeral stria present as a median fragment; dorsal elytral striae vaguely impressed, almost obliterated under coarse rugulose-lacunose punctuation, only first and second dorsal striae distinguishable, not reaching elytral midpoint apically, third and fourth striae faint, shorter than first and second; sutural elytral stria faintly impressed, abbreviated at basal tenth, complete to apex, continuous with apical elytral stria; entire elytral disc (with exception of elytral humeri) rugulose-lacunose.

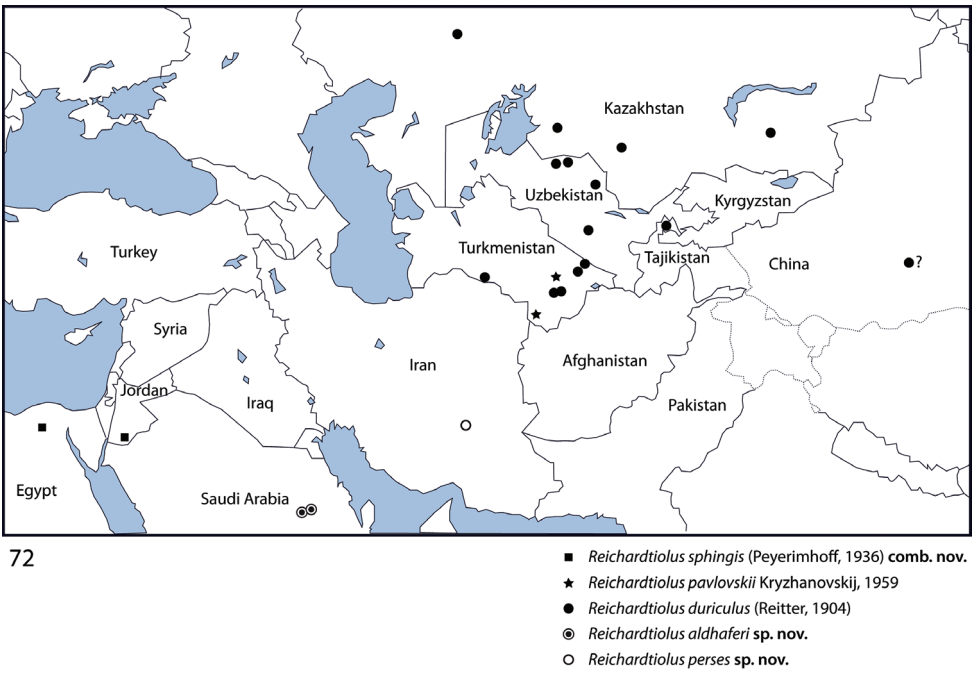
Propygidium largely covered by elytra; its punctuation similar to that of elytral disc; pygidium also densely and coarsely punctate; punctures with minuscule setae.

Anterior margin of median portion of prosternum (Fig. 68) projected medially, setose; prosternal foveae absent; marginal prosternal stria present laterally and as extremely short apical rudiment; prosternal apophysis constricted between procoxae, rugulose-lacunose, setose, prosternal process thence strongly compressed, knife-like, setose, surface imbricate, dorso-medially with numerous setiferous punctures; vestiges of carinal prosternal striae present on prosternal apophysis; lateral prosternal striae present as faint rudiments, almost invisible.

Anterior margin of mesoventrite with slight median projection; discal marginal mesoventral stria complete; disc of mesoventrite convex, rugulose-lacunose; meso-metaventral suture straight, thin; meso-metaventral sutural stria undulate; intercoxal disc of metaventrite (Fig. 69) depressed medially; with sparser and finer punctuation than that of mesoventrite, punctures separated by two-three times their diameter; lateral metaventral stria straight, shortened; lateral disc of metaventrite slightly excavate, with dense deep setiferous punctures; metepisternum (Fig. 69) with similar setiferous punctures; fused metepimeron with sparser punctuation; lateral metepisternal stria complete, deeply impressed.

Intercoxal disc of first abdominal ventrite completely striate laterally; completely covered with punctuation; punctures similar to those of disc of metaventrite.

Protibia (Figs 64, 67) dilated, outer margin with three large widely-spaced distal teeth topped by large triangular denticle, diminishing in size in proximal direction, followed by two smaller proximal denticles; setae of outer row thin, sparse and short; setae of median row similar to those of outer row; protarsal groove shallow; anterior protibial stria carinate, almost complete; protibial spur small, straight, growing out near tarsal insertion; outer part of posterior surface of protibia (Fig. 67) almost smooth,



Figures 72. Distributional map of *Reichardtiolus* Kryzhanovskij, 1959.

only with scattered microscopic denticles, demarcation line between outer and median of posterior surface non-existent; posterior protibial stria absent, near inner protibial margin a dense row of strongly sclerotized long setae present; inner margin with sparser row of thinner setae.

Mesotibia slightly thickened, outer margin with row of approximately ten long denticles increased in size apically; setae of outer row dense and long, strongly sclerotized, longer than denticles on outer margin; setae of median row absent; posterior mesotibial stria absent; anterior surface of mesotibia with additional two-three dense rows of short denticles; anterior mesotibial stria complete, terminating in several minute denticles; mesotibial spur short; apical margin of mesotibia with a row of about five short denticles; first and second tarsomere ventrally with four long, strongly sclerotized setae; third and fourth tarsomeres with only two such setae; fifth tarsomere devoid of setae ventrally; claws of apical tarsomere slightly bent, longer than tarsomere itself; metatibia (Fig. 70) much more thickened and dilated than mesotibia, outer margin and posterior surface similar to that of mesotibia; anterior surface of metatibia completely covered with six-seven rows of short, stout denticles (Fig. 71).

Male unknown.

Differential diagnosis. Externally somewhat similar to its congeners, it is, however, the most readily distinguishable species of the five. Body (Figs 62–63) larger than in all other congeners (up to 4.25 mm in *R. pavlovskii*, whereas other *Reichardtiolus* species attain maximal body length of 3.75 mm), cuticle dark brown to black, en-

tire dorsal surface rugulose-lacunose, whereas the dorsal surface of the other species is mostly chestnut brown and punctate, never rugulose-lacunose. Dorsal elytral striae (Fig. 62) of *R. pavlovskii* are vaguely impressed, almost obliterated under coarse rugulose-lacunose punctuation, only first and second dorsal striae distinguishable, while with the rest of congeners they are usually distinct. This species differs likewise from the rest of its congeners by the structure of the prosternal keel (compare Figs 10–11, 36, 50 and 68), which is projected medially, strongly compressed, almost knife-like, lacking foveae, and with only vestigial striae. *R. pavlovskii* also differs from the other species by the lateral disc of the metaventrite and fused metepisternum (Fig. 69) that are covered with almost confluent setiferous punctures, whereas the punctures are not confluent in *R. duriculus*, *R. perses*, *R. aldhaferi* or *R. sphingis*. The protibia (Figs 64 and 67) is similar to the other three species, but adorned with three short teeth topped by acute large triangular denticle (instead of two) followed by one shorter denticle entombed in protibial margin and one more microscopic denticle. The mesotibia on its anterior surface has an additional two-three dense rows of short denticles instead of the single row present in *R. duriculus*, *R. sphingis*, *R. perses* and *R. aldhaferi*; the metatibia (Figs 70–71) is much more thickened and dilated than those of the other four species; the anterior surface of metatibia has six-seven rows of short stout denticles as opposed to only two rows in *R. duriculus*, *R. sphingis*, *R. perses* and *R. aldhaferi*. Unfortunately, the only examined specimen is a female so the male genitalia could not be compared to those of other species.

Biology. Found in the sand under *Tamarix* (Kryzhanovskij & Reichardt, 1976).

Distribution. So far known only from two places in Turkmenistan: about 40 km north of Mary, eastern Turkmenistan and Badkhyz Nature Reserve, southeastern Turkmenistan (Fig. 72).

Remarks. Kryzhanovskij (1959), in his original description, omitted the character of the prosternal striae, and in the Fauna USSR (Kryzhanovskij and Reichardt 1976) he provided a brief re-description of this species but omitted the prosternum altogether, pointing only to the greater size and surface of the dorsal side of body as distinguishing characters for separating *Reichardtiolus duriculus* from *R. pavlovskii*. *R. pavlovskii* is, according to Kryzhanovskij in Kryzhanovskij and Reichardt (1976) known only from two females and I have only examined one of them, the holotype. The repository of the second specimen of this rare species is unknown. Although *R. pavlovskii* is morphologically rather different from the other species of the genus, I am hesitant to erect a new genus for it, especially since no male is available and the male terminalia could not be examined.

Key to the species of the genus *Reichardtiolus*

1(2) Metatibia on anterior surface (Fig. 71) with more than 5 dense rows of tiny denticles; protibia on outer margin with three short teeth topped by denticle (Fig. 64), followed by one more small tooth embedded in the outer margin

topped by a denticle and a minuscule denticle; a large species (4.25 mm) (Turkmenistan) ..... ***Reichardtiolus pavlovskii*** **(Kryzhanovskij, 1959)**

2(1) Metatibia on anterior surface with one or two sparse rows of tiny denticles (for fig. see Lackner 2010: fig 602); protibia on outer margin with two short teeth topped by denticle (for fig. see Lackner 2010: fig 603), followed by one more small tooth embedded in the prosternal margin topped by a denticle and a minuscule denticle; smaller species (up to 3.80 mm).

3(4) Mentum almost without emargination on anterior margin (Fig. 4), 8th tergite apically almost straight (Fig. 15); spiculum gastrale apically only faintly inwardly arcuate (Fig. 19), species from middle Asia ..... ***R. duriculus*** **(Reitter, 1904)**

4(3) Mentum anteriorly with moderately deep to deep emargination (Fig. 5), 8th tergite apically deeply emarginate (see for example Fig. 39); spiculum gastrale apically strongly inwardly arcuate (see for example Fig. 43); species from Near East, Iran.

5(6) Aedeagus strongly curved from lateral view (Fig. 46), thickened medially (Fig. 45); species from Saudi Arabia ..... ***R. aldhaferi*** **sp. n.**

6(5) Aedeagus only moderately curved from lateral view (Figs 33, 61), not particularly thickened medially (Figs 32, 60); species from Egypt, Jordan and SW Iran

7(8) Basal margin of 10th tergite moderately inwardly arcuate, without a prominent incision (Fig. 57), both tips of apical end of spiculum gastrale without strongly sclerotized parts (Fig. 54), sclerotization of 9th tergite medially divided (Fig. 57), species from SW Iran ..... ***R. perses*** **sp. n.**

8(7) 10th tergite on basal margin with median incision (Fig. 26), both tips of apical end of spiculum gastrale with strongly sclerotized parts (Fig. 29), sclerotization of 9th tergite undivided medially (Fig. 26), species from N Egypt and S Jordan ..... ***R. sphingis*** **(Peyerimhoff, 1932)**

Discussion

Reichardtiolus is a small psammophilous Saprininae genus currently comprising five species: *R. duriculus*, *R. sphingis*, *R. aldhaferi*, *R. perses* and *R. pavlovskii*. Although the four former species are morphologically very similar and undoubtedly related, the latter species *R. pavlovskii* is rather different from the rest and characterized by several autapomorphies, e.g. rudimentary sets of prosternal striae, absence of prosternal foveae, and more than five rows of densely set short denticles on the anterior surface of metatibia. Its protibia is also different from those of *R. duriculus*, *R. sphingis*, *R. perses* or *R. aldhaferi* by having an extra tooth on its outer margin. The four morphologically similar species apparently represent allopatric congeners all sharing a rather recent common ancestor, since they only differ in minute details most evident in their male genitalia. It is possible that their common ancestor came from the deserts of middle Asia, and subsequently speciated in the arid regions of North Africa, Near East, and Iran in search for new habitats as a form of adaptive radiation. All five species seem to

be well adapted to the psammophilous way of life with thickened femora and tibiae, enlarged protibiae with large triangular teeth each topped by a denticle, as well as having the underside of the body covered with vestiture.

Phylogenetically speaking, the type species of the genus has been recovered in the recently performed cladistic analysis of the author (Lackner, unpublished) as a member of a large unresolved clade of taxa that all share a single unique synapomorphy of a single, stipe-shaped vesicle inside the internal-distal part of the antennal club, as well as several other, weaker synapomorphies. However, the species *R. pavlovskii*, which was also included in the analysis, has been recovered rather distant from the type species of the genus, *R. duriculus*. Because of the low resolution of the morphology-based cladogram, and absence of a male specimen of *R. pavlovskii* I decided not to alter the generic rank of the latter species. The members of the genus *Reichardtiolus* cover a rather vast area (Fig. 72) from the Chinese Xinjiang province in the east to the Egyptian locality in the west, from the Kazakh localities in the north to the Saudi Arabian localities in the south. Such a vast area likely houses further undescribed species of *Reichardtiolus* and it is hoped that this study shall encourage their discovery by fellow entomologists.

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References

Kryzhanovskij OL (1959) Novyy vid psammofil'nogo zhuka (Coleoptera, Histeridae) iz Turkmenskoy SSR. (New species of a psammophilous beetle from Turkmen SSR). Entomologicheskoe Obozrenie 38: 216–218. [in Russian, English summary]

Kryzhanovskij OL, Reichardt AN (1976) Zhuki Nadsemeystva Histeroidea (semeystva Sphaeritidae, Histeridae, Synteliidae). [Beetles of the superfamily Histeroidea (families Sphaeritidae, Histeridae, Syntelidae)]. In: Fauna SSSR, Zhestokrylye, Vyp. 4. Nauka, Leningrad, 434 pp. [in Russian]

Lackner T (2009a) Revision of the genus *Saprinillus*, with description of a new species (Coleoptera: Histeridae). Folia Heyrovskyana, Series A 16(4): 107–118.

Lackner T (2009b) Revision of the genus *Zorius* (Coleoptera: Histeridae). Folia Heyrovskyana, Series A 16(4): 119–129.

Lackner T (2009c) Revision of the genus *Terametopon*, with description of new species (Coleoptera: Histeridae). Folia Heyrovskyana, Series A 17(2): 43–72.

Lackner T (2010) Review of the Palaearctic genera of Saprininae (Coleoptera: Histeridae). Acta Entomologica Musei Nationalis Pragae 50 (Supplementum): 1–254.

Lackner T (2011a) On the identity of *Chalcionellus orcinus* Reichardt, 1932 and Chalcionellus libanicola (Marseul, 1870) (Coleoptera: Histeridae). Acta Entomologica Musei Nationalis Pragae 51(2): 505–515.

Lackner T (2011b) Revision of the genus *Alienocacculus* Kanaar 2008 (Coleoptera: Histeridae: Saprininae). Folia Heyrovskyana 19(1-4): 139–157.

Lackner T (2012) Revision of the genus *Xenonychus* Wollaston, 1864. Acta Entomologica Musei Nationalis Pragae 52(1): 147–159.

Lackner T (2013a) Revision of the genus *Ctenophilothis* Kryzhanovskij, 1987 (Coleoptera: Histeridae: Saprininae). Zootaxa 3691(2): 273–282. doi: 10.11646/zootaxa.3691.2.6

Lackner T (2013b) Elevation of *Pilisaprinus* to full generic status with notes on the evolution of termitoxeny in the Saprininae (Coleoptera: Histeridae). Acta Entomologica Musei Nationalis Pragae 53(2): 623–632.

Lackner T, Gomy Y (2013) *Malagasyprinus*, a new genus of the Saprininae subfamily from Madagascar with description of two new species (Coleoptera: Histeridae: Saprininae) (First contribution to the knowledge of the Histeridae of Madagascar). Zookeys 333: 55–76. doi: 10.3897/zookeys.333.5909

Mazur S (1984) A world catalogue of Histeridae. Polskie Pismo Entomologiczne 54(3–4): 1–376.

Mazur S (1997) A world catalogue of the Histeridae (Coleoptera: Histeridae). Genus, Supplement: 1–373.

Mazur S (2004) Family Histeridae. In: Löbl I, Smetana A (Eds) Catalogue of Palaearctic Coleoptera, Vol. 2, Hydrophiloidea-Histeroidea-Staphylinoidea. Apollo Books, Stenstrup, 68–102.

Mazur S (2011) A concise catalogue of the Histeridae (Coleoptera). Warsaw University of Life Sciences, SGGW Press, Warsaw, 332 pp.

Ôhara M (1994) A revision of the superfamily Histeroidea of Japan (Coleoptera). Insecta Matsumurana (N. S.) 51: 1–238.

Peyerimhoff P (1936) Les Saprinines Sabulicoles du Nord de l'Afrique (Coleoptera: Histeridae). Bulletin de la Société Entomologique d'Égypte 20: 213–228.

Tishechkin AK, Lackner T (2012) Revision of the genus *Eremosaprinus* Ross with elevation of *Erebidus* Reichardt to genus and description of new species (Coleoptera: Histeridae: Saprininae). Folia Heyrovskyana 20(1-2): 1–53.

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Darwin's legacy to rove beetles (Coleoptera, Staphylinidae): A new genus and a new species, including materials collected on the Beagle's voyage

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Abstract

A species of xanthopygine rove beetles is described and figured here as *Darwinilus sedarisi* **gen. n.** and **sp. n.** The holotype was collected by Charles Darwin in Bahía Blanca, Argentina on the Beagle's voyage. The contributions of Charles Darwin to rove beetle systematics are summarized briefly.

Keywords

Argentina, Neotropical, South America, Staphylininae, Staphylinini, Xanthopygina

Introduction

Charles Darwin was an avid beetle collector and his contributions to the study of entomology have been extensive (Stephens 1827–1845; Waterhouse 1879; Champion 1918; Kritsky 1981; Smith 1987). Darwin's collecting efforts on the Beagle's voyage (1831–1836) were important because he brought back to the United Kingdom specimens from places that had not been sampled before. Darwin kept meticulous notes on the specimens he collected and those notes are known as "Insect Notes" (kept at the

Entomology Library of the Natural History Museum, London) and "Insects in Spirits of Wine" (kept at the Cambridge University Library). Smith (1987) provided annotated versions of those notes giving details on the taxonomy of the specimens collected and whether or not these specimens still exist in collections.

Based on the annotated Insect Notes (Smith 1987) we know that Darwin had at least 14 collecting events that included rove beetles (Table 1). These include species in the subfamilies Aleocharinae, Microsilphinae, Oxytelinae, Pselaphinae, Scaphidiinae, Scydmaeninae and Staphylininae. Until now, five new species of rove beetles had been described based on Darwin materials and most of those are still considered valid species. Four more species of rove beetles are currently known from Darwin's collecting efforts but the type materials of these species did not include Darwin's specimens. Additionally, Smith (1987) did not find specimens for some of the collecting events that included rove beetles.

Over the last several years, I have been working towards revising all genera in the rove beetle subtribe Xanthopygina, a group of large and colorful rove beetles distributed in the New World tropics (Chatzimanolis 2014). While examining specimens for the review of *Trigonopselaphus* Gemminger and Harold (Chatzimanolis in preparation), I noticed a specimen borrowed from the Natural History Museum (London) that had serrate antennae, an atypical morphological feature in rove beetles. Upon further inspection, I realized that the specimen belonged to an undescribed genus and that it was Charles Darwin who had collected it on the Beagle's voyage. In this paper I describe this and one additional conspecific specimen as a new genus and species of Xanthopygina, the second new genus of rove beetles to be described from Beagle's expedition materials.

Materials and methods

Specimens were studied using an Olympus SZX10 dissecting microscope. Specimens examined were loaned from the Natural History Museum, London (BMNH; Roger Booth) and the Museum für Naturkunde der Humboldt Universität (ZMHB; M. Uhlig, B. Jaeger). The 181-year old Darwin specimen was relaxed carefully using the steam method described in a Natural History Museum (London) blog post by curator Beulah Garner, (http://www.nhm.ac.uk/natureplus/blogs/beetles/2011/11/05/steamy-beetles-or-whats-the-point). The paratype was already dissected when I received the specimen from ZMHB. Some aspects of the morphology (e.g., extensive details on mouthparts) were not described due to the fragile state (and at the same time high scientific value) of both specimens. Photographs were taken using a Visionary Digital Passport system with a Canon EOS 40D. Final images were automontaged using Helicon Focus 4.2.9 Pro (http://www.heliconsoft.com/heliconfocus.html). Total length of the specimens is measured from the anterior margin of frons to the posterior margin of segment VIII; width: length measurements were made on the widest: longest part of the structure. Measurements were made with an ocular micrometer. The com-

Table 1. Checklist of rove beetles collected by Charles Darwin on the Beagle's Voyage. The list has been extracted from Smith (1987) with updates on the taxonomy. Date refers to the date of the collecting event as documented by Darwin. Months are given in roman numerals (when available). Specimen no. refers to the collecting event number given by Darwin.

Date	No.	Locality	Subfamily: Tribe: Subtribe	Species	Comments	Reference
1832-ii-16	229	St. Paul's Rocks, Brazil			Specimen not found; hypothesized by Smith (1987) to be either *Philonthus* or *Quedius*.	Smith (1987)
1832-iv	415	Rio de Janeiro, Brazil	Scaphidiinae: Scaphisomatini	*Scaphisoma elongatum* Waterhouse, 1879	Species described from Darwin specimen.	Waterhouse (1879)
1832-ix	708	Bahía Blanca, Argentina	Staphylininae: Staphylinini: Xanthopygina	*Darwinilus sedarisi* Chatzimanolis, gen. n. and sp. n.	Specimen listed as "not found" in Smith (1987); genus and species described from Darwin specimen.	this paper
1832-xii-20	906	Navarin Is., Chile	Aleocharinae: Oxypodini	*Nordenskjoldella flavitarsis* Enderlein, 1912	Type material not based on Darwin specimen.	Enderlein (1912); Champion (1918)
1833-iii	1151	Tierra del Fuego	Aleocharinae: Homalotini	*Leptusa* (*Halmaeusa*) *atriceps* (Waterhouse, 1875)	Originally described as *Phytosus darwini* Waterhouse 1879 and was based on Darwin specimen.	Waterhouse (1879); Steel (1964)
1834-i	2002	Port St Julian [Puerto San Julián], Argentina			Specimens not found.	Smith (1987)
1834	2369	Archipelago of Chiloé, Chile	Microsilphinae	*Microsilpha ocelligera* (Champion, 1918)	Originally described in *Micragyrtes;* type materials based on Darwin materials.	Champion (1918); Newton and Thayer (1995)
1834	2371	Archipelago of Chiloé, Chiloé Is., Chile	Aleocharinae: Oxypodini	*Polylobus darwini* Bernhauer, 1935	Species described from Darwin specimen.	Bernhauer (1935)
1834-xii	2424	Archipelago of Chronos, Chile			Specimens not found; Darwin commented that "Pselaphidae and small Staphylinidae the most abundant insects"	Smith (1987)
1835	3426	Galapagos Archipelago, San Cristóbal Is., Ecuador	Staphylininae: Staphylinini	*Creophilus galapagensis* Clarke, 2011	Type material not based on Darwin specimen.	Clarke (2011)
1835-ii	3445	Hobart Town, Tasmania, Australia			Specimens not found; hypothesized by P. Hammond to be *Creophilus erythrocephalus* F.	Smith (1987)
1835-ii	3524	Hobart Town, Tasmania, Australia	Scaphidiinae: Scaphisomatini	*Scaphisoma instabile* Lea, 1926	Lectotype not based on Darwin specimen.	Lea (1926); Löbl (1977)
1836-vii	3730	St. Helena	Scydmaeninae: Cyrtoscydmini	"*Anthicus wollastoni*" Waterhouse, 1879	The type was based on Darwin material; not Anthicidae but Scydmaeninae (in *Euconnus*) according to Smith (1987).	Waterhouse (1879); Champion (1895)
1836-vii	3730	St. Helena	Oxytelinae: Oxytelini	*Oxytelus alutaceifrons* Wollaston, 1877	Type material not based on Darwin specimen.	Wollaston (1877); Waterhouse (1879)

parison of the length of the parameres and the median lobe excludes the bulbous basal portion of the median lobe. For type label data, the slash "/" separates different labels. Morphological terminology follows Ashe and Chatzimanolis (2003) and other recent revision of Xanthopygina (Chatzimanolis 2004, 2008, 2012; Chatzimanolis and Ashe 2009). In this paper I follow the phylogenetic species concept as outlined by Wheeler and Platnick (2000).

Taxonomy

Family Staphylinidae Latreille, 1802
Subfamily Staphylininae Latreille, 1802
Tribe Staphylinini Latreille, 1802
Subtribe Xanthopygina Sharp, 1884

***Darwinilus* Chatzimanolis, gen. n.**
http://zoobank.org/BD229C1A-4D45-4BF5-B780-52CA5C2720B2
http://species-id.net/wiki/Darwinilus

Type species. *Darwinilus sedarisi* Chatzimanolis, sp. n.

Diagnosis. *Darwinilus* can be distinguished from all other Xanthopygina genera by the combination of the following characters: a) serrate antennae (antennomeres 5–11; antennomeres 6–10 asymmetrical in *Terataki* Chatzimanolis, *Triacrus* Nordmann and *Trigonopselaphus* but not as in *Darwinilus*); b) clypeus with shallow emargination; c) protibia strongly curved and d) absence of porose structure on abdominal sternite VII in males. *Darwinilus* is probably closely related to the genera *Terataki* Chatzimanolis and/or *Haematodes* Laporte and *Weiserianum* Bernhauer but can be easily distinguished from these genera by the presence of serrate antennae in *Darwinilus* and the lack of porose structure on abdominal sternite VII in males (present in *Terataki*, *Haematodes* and *Weiserianum*).

Description. Habitus as in Fig 1, body large, robust. Head hexagonal in shape (Figs 2–3), widest at temples. Eyes medium-sized, positioned anteriorly, distance between eyes as wide as twice length of eye. Postoccipital suture and ventral basal ridge present; presence of infraorbital ridge not clear but ridge situated between postmandibular ridge and gular suture extends from posterior to middle part of gena; postmandibular ridge present and prominent; gular sutures converging medially; without neck (no nuchal ridge). Epicranium with large prominent macrosetae around lateral margins. Anteclypeus expanded, clypeus with small v-shaped emargination medially. Antennae serrate, 11–segmented; antennomeres 1–3 with several rows of macrosetae; antennomeres 4–11 covered with microtrichiae. Mouthparts with labrum medially emarginate to its base. Mandibles curved, elongate, symmetrical, with prominent fold extending from base to near middle; right mandible with at least one prominent tooth; prostheca setose. Maxilla with galea and lacinia setose; maxillary palpi 4-segmented;

palpomeres with several large setae; P_1 short; P_2– P_4 elongate; P_2– P_3 curved, wider distally; P_2 2.2 times as long as P_1; P_3 shorter than P_2; P_4 subequal to P_3, rounded apically. Labium with mentum having two anterolateral setae on each side; ligula short, entire; labial palpi 3-segmented; P_1 subequal to P_2; P_2 widest anteriorly, with many large setae; P_3 elongate, longer than P_2, securiform [but not as dilated as in *Zackfalinus* Chatzimanolis or *Dysanellus* Bernhauer; see Chatzimanolis 2012]. Pronotum slightly wider than head; with small translucent postcoxal process; pronotal hypomeron expanded; superior and inferior marginal lines of hypomeron separate throughout their length and superior line fully visible from above (typical of Xanthopygina). Anterolateral corners of pronotum prominent. Pronotum (Fig 4) with microsculpture and punctures of various sizes; with prominent macrosetae along margins. Basisternum with transverse microsculpture and various setae; anterior marginal depression present; sternacostal ridge present; furcasternum without carina. Elytra (Fig 5) longer than pronotum; with long yellow macrosetae, especially prominent at lateral and posterior margins. Elytra depressed near mesoscutellum. Hind wings fully developed. Mesoventrite without median carina or mesoventral process; metaventrite with transverse microsculpture and uniform medium-sized punctation; metaventral process small, triangular. Legs with tarsal segmentation 5-5-5; tibia with ctenidium and several rows of small spurs; meso- and metatibia with two long apical spurs, spurs as long as basitarsus; protibia strongly curved; meso- and metatibia slightly curved. Protarsus enlarged in males [no females are known]; meso- and metatarsi not enlarged; empodium with two setae. Abdomen (Figs 6–7) with abdominal tergites III–V with anterior basal carina but without curved (arch-like) ridge and without accessory basal lines. Abdominal sternite VII in males without porose structure. Male genitalia (Figs 8–9) typical of Xanthopygina; aedeagus with long median lobe; paramere partially divided distally.

Etymology. The genus name is derived from the word "Darwin" in honor of Charles Darwin who collected the beetle during the voyage of the Beagle. The name is masculine.

***Darwinilus sedarisi* Chatzimanolis, sp. n.**
http://zoobank.org/6AB0C47D-5A4B-4D59-AB99-E188FB1E95D2
http://species-id.net/wiki/Darwinilus_sedarisi
Figs 1–10

Type locality. Bahía Blanca, Argentina.

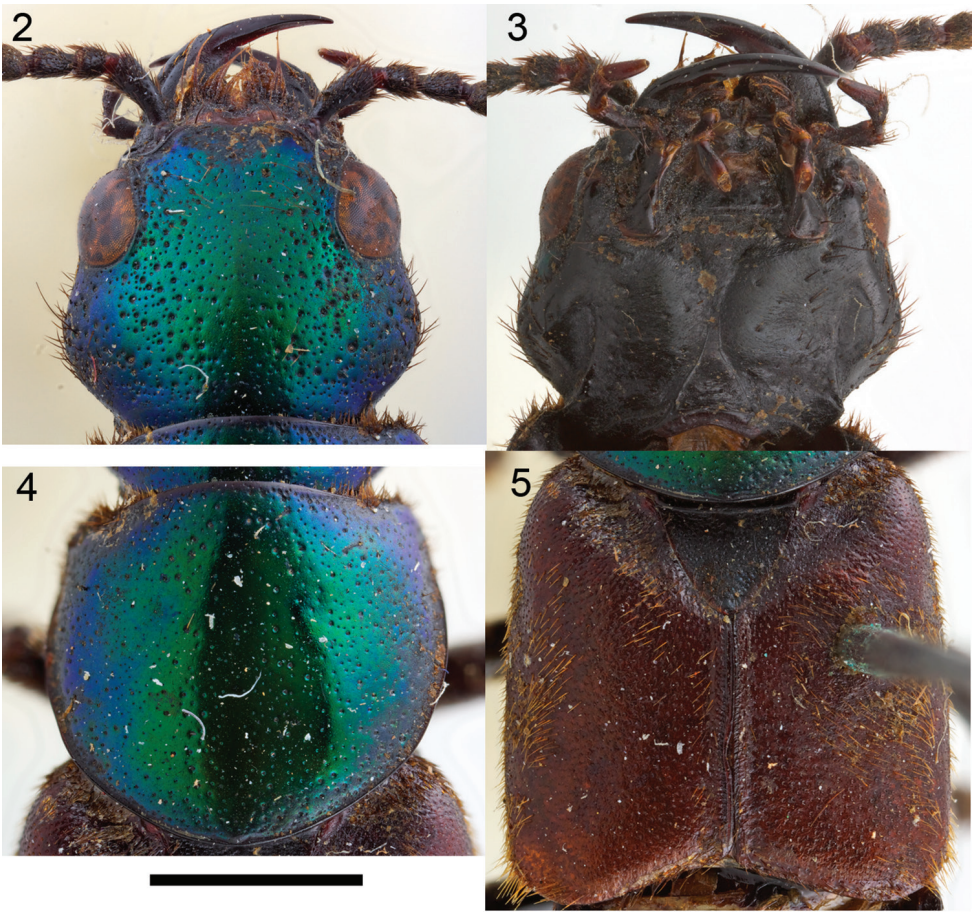
Holotype. Male, dry pinned, with labels as follows: "B. Blanca" / "708" / "Darwin Coll. 1885.-119." / "Bahía Blanca, Argentina. C. Darwin." / "?*Trigonopselaphus* A. Solodovnikov det. 2007" / "Holotype *Darwinilus sedarisi* Chatzimanolis des. Chatzimanolis 2013". Darwin arrived on Bahía Blanca on September 6, 1832 and departed on October 17, 1832 according to Barlow (1967). The specimen was collected in September according to the Insect Notes that Darwin kept (Smith 1987). The holotype shows evidence of prior damage since several body parts have been reattached with non

Figure 1. Habitus of the holotype of *Darwinilus sedarisi* Chatzimanolis, sp. n. Total length = 21.5 mm Image Copyright Natural History Museum (London).

water-soluble glue. Deposited in BMNH. **Paratype** (1) male: **Argentina**, Córdoba, Río Cuarto, Breuer coll. (ZMHB).

Diagnosis. As for the genus.

Description. Body length 20.0–21.5 mm. Coloration of head and pronotum metallic green with blue-purple overtones near margins. Elytra light brown. Mouthparts, mesoscutellum, legs, abdomen and ventral surface of body dark brown-black. Antennae dark brown except antennomeres 4–7 appearing yellowish brown due to the presence of

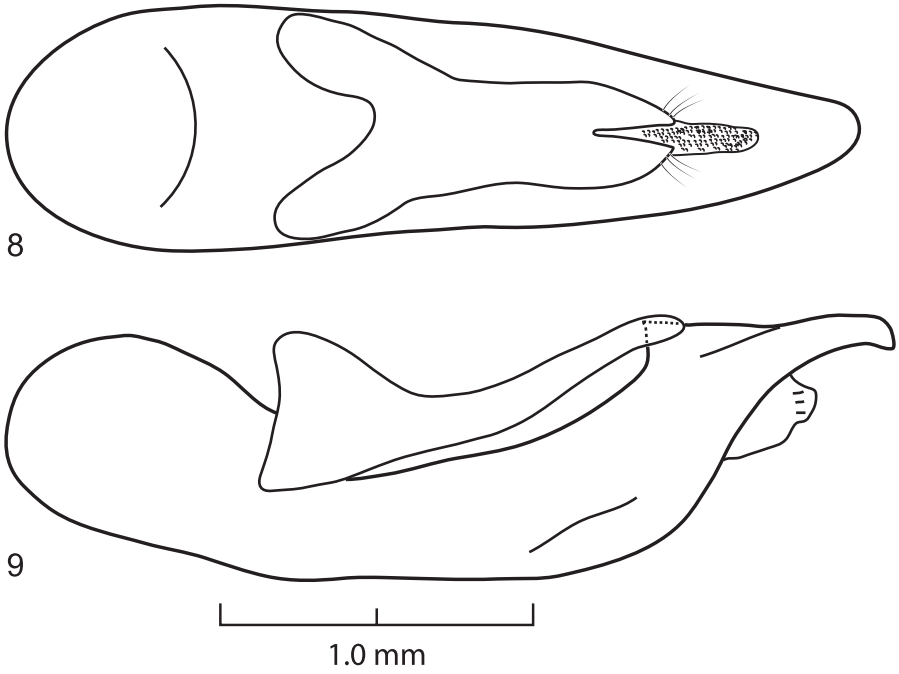


Figures 2–5. Head and thorax of the holotype of *Darwinilus sedarisi* Chatzimanolis, sp. n. **2** Head, dorsal view **3** Head, ventral view **4** Pronotum **5** Elytra. Scale = 2.2 mm Image Copyright Natural History Museum (London).

yellow microtrichiae. Head slightly transverse, width : length ratio = 1.23. Dorsal surface of head with uniform dense polygon-shaped microsculpture, small punctures interspersed and medium to large size punctures throughout except medially. Ventral surface of head with transverse microsculpture, micropunctures and few large punctures along borders of gula and directly posterior to mandibles. Antennomeres 1–3 longer than wide; antennomere 4 shorter but wider than 3; antennomere 5 narrower than 6; antennomeres 6–7 subequal in size; antennomere 8 slightly wider than 7; antennomeres 8–10 subequal in size; antennomeres 5–11 serrate. Pronotum width : length ratio = 1.08, widest medially; with uniform dense polygon-shaped microsculpture; small punctures interspersed and medium to large size punctures throughout except medial line; medium to large size punctures also present around margin of pronotum but not in rows as is typical in other Xanthopygina. Mesoscutellum with polygon-shaped microsculp-

Figures 6–7. Abdomen of the holotype of *Darwinilus sedarisi* Chatzimanolis, sp. n. **6** Dorsal view **7** Ventral view. Scale = 3 mm Image Copyright Natural History Museum (London).

ture and uniform small almost confluent punctures. Elytra longer than pronotum; with dense polygon-shaped microsculpture and uniform punctation consisted of medium-sized almost confluent punctures; sutures of elytra with 2–3 rows of micropunctures on each side. Abdominal tergites with dense transverse microsculpture and uniform small-sized punctures; punctures almost confluent except punctation less dense medially on tergites III–IV. Sternum with uniform dense punctuation consisted of small punctures; additional irregular row of larger punctures near posterior margin on sternites V–VII; sternum with transverse microsculpture. Male secondary sexual structures: posterior border of sternite VIII having deep V-shaped emargination medially; sternite IX with shallow U-shaped emargination. Aedeagus as in Figs 8–9; paramere separated anteriorly into two lobes; lobes slightly asymmetrical; paramere much shorter and narrower than median lobe; paramere without peg setae; in dorsal view each paramere lobe converging to rounded apex; in lateral view paramere curved upwards. Median lobe in dorsal view wide, converging to rounded apex; with single large dorsal tooth; in lateral view median lobe curved upwards to prominent tooth, then becoming much narrower and slightly curved downwards to rounded apex.



Figures 8–9. Aedeagus of *Darwinilus sedarisi* Chatzimanolis, sp. n. **8** Dorsal view **9** Lateral view.

Figure 10. Original BMNH labels for the holotype of *Darwinilus sedarisi* Chatzimanolis, sp. n. Image Copyright Natural History Museum (London).

Etymology. The species is named in honor of Mr David Sedaris, a prolific writer, as an appreciation for his fascination with the natural world. I spent many hours listening to Mr Sedaris' audiobooks while preparing the specimens and the figures for this and other manuscripts.

Distribution. Known from Bahía Blanca, Buenos Aires and Río Cuarto, Córdoba in Argentina.

Habitat. Unknown; the climate in the areas mentioned above is humid subtropical to humid temperate. However, agricultural fields have replaced the original habitat in these localities.

Remarks. It is rather remarkable that only two specimens are known for such a large species. I have examined the rove beetle collections of most major museums in

North America and Europe but unfortunately I was not able to locate any additional specimens. One explanation might be that this species lives in refuse piles of ants or other Hymenoptera (see below for further discussion).

Discussion

The Darwin specimen described in this paper as the holotype of *Darwinilus sedarisi* was given the specimen number 708 in the Insect Notes held by Darwin and Syms Covington (Darwin's servant). Until now, this specimen was considered lost (or "not found") according to Smith (1987) in the BMNH collection. Alternatively, Smith hypothesized that specimen 708 (or perhaps 3445, see Table 1) could have been present in the Field Museum (FMNH), Chicago, given that Kritsky (1981) mentioned a Darwin rove beetle specimen was present there. However, the presence of such specimen in FMNH is unlikely given that several Coleoptera curators (H. Dybas, H. Nelson, A. Newton, M. Thayer, R. Wenzel; Newton personal communication) were not aware of any such specimens. It is likely that several of the Darwin specimens considered "not found" in Table 1 have been curated to other parts of the collection in BMNH, presumably to where they taxonomically belong. However, that was not the case for specimen 708, which was found among unsorted Staphylinidae materials by my colleague A. Solodovnikov (personal communication). He transferred the specimen to the unidentified materials of the genus *Trigonopselaphus* as the best tentative placement, an act that allowed me to discover this specimen later on when I borrowed the *Trigonopselaphus* specimens from BMNH.

Darwinilus is superficially similar to *Trigonopselaphus* (due to the large habitus) but it is probably more closely related to the newly erected genus *Terataki* (Chatzimanolis 2013) and/or the genera *Haematodes* and *Weiserianum. Darwinilus* shares with *Terataki* and *Haematodes* similarities in the morphology of the head (hexagonal shape, position of ridges and sutures ventrally, and mouthpart morphology) and the partially divided parameres of the aedeagus. Given the fragile state of both specimens used to describe *Darwinilus*, more specimens are required to add this taxon to a molecular/morphological phylogeny of the subtribe (Chatzimanolis in preparation).

No data were available regarding the natural history of *D. sedarisi*. The genus *Weiserianum*, hypothesized to be related to *Darwinilus*, is known to be a myrmecophile (leafcutter ants; Scheerpeltz 1936). A few other large South American xanthopygines are known to occur with social Hymenoptera other than ants such as the species *Triacrus dilatus* Nordmann (in debris piles of *Stenopolybia vicina* (de Saussure), a vespid wasp; Wasmann 1902), but clearly natural history observations are needed to understand the biology of *D. sedarisi*. Future collecting expeditions should focus on gathering natural history information for *D. sedarisi* as well as better defining its distribution range. Presently, *D. sedarisi* is known from two localities (Bahía Blanca and Río Cuarto) in Argentina separated by several hundred kilometers. Although the exact date for the collecting event in Río Cuarto by Breuer is not known, it took place before 1935 since the Breuer collection was already in ZMHB by that time (Jaeger personal com-

munication; Horn and Kahle 1935:30). Much of the area between Bahía Blanca and Río Cuarto has been converted into agricultural fields and it is questionable if that is a suitable habitat for the species. One of course hopes that a newly described species is not already extinct. Perhaps more specimens of *Darwinilus* remain unsorted in Natural History Museums in North America, Europe or South America, and the publication of this paper will bring these specimens to light.

Acknowledgments

I am grateful to my colleague A. Solodovnikov who mentioned in early 2008 that there was a "Darwin xanthopygine" in BMNH. I thank Al Newton for the notes on rove beetle species described by Darwin and anecdotes regarding a Field Museum specimen. I am in debt to the curators and collection managers in BMNH and ZMHB for the loan of specimens and their hospitality. I thank Michael S. Engel, Cheryl Murphy, Alexey Solodovnikov and an anonymous reviewer for comments on a previous version of the manuscript. The Natural History Museum of London kindly allowed me to photograph the holotype, they retain the copyright of the photographs.

References

Ashe JS, Chatzimanolis S (2003) A revision of the genus *Elmas* Blackwelder, 1952 (Coleoptera: Staphylinidae), with a preliminary reconstructed phylogeny of the species. Scientific Papers of the Natural History Museum of the University of Kansas 28: 1–41.

Barlow N (1967) Darwin and Henslow. The growth of an idea. Letters 1831–1860. John Murray, London, 251 pp.

Bernhauer M (1935) Neue Staphyliniden (Col.) aus Südamerica. Stylops 4: 90–96.

Champion GC (1895) *Anthicus wollastoni*, F. Waterh[ouse]. Entomologist's monthly Magazine 31: 75.

Champion GC (1918) Notes on various South American Coleoptera collected by Charles Darwin during the voyage of the "Beagle", with descriptions of new genera and species. Entomologist's monthly Magazine 54: 43–55.

Chatzimanolis S, Ashe JS (2009) A revision of the neotropical genus *Ocyolinus* (Coleoptera: Staphylinidae: Staphylinini). Zootaxa 2162: 1–23

Chatzimanolis S (2004) A revision of the neotropical genus *Nordus* Blackwelder (Coleoptera: Staphylinidae: Xanthopygina). Entomologische Abhandlungen 62(1): 3–64.

Chatzimanolis S (2008) A revision of the neotropical beetle genus *Isanopus* (Coleoptera: Staphylinidae: Staphylinini). Journal of Natural History 42(25–28): 1765–1792. doi: 10.1080/00222930802124057

Chatzimanolis S (2012) *Zackfalinus*, a new genus of Xanthopygina (Coleoptera: Staphylinidae: Staphylinini) with description of 20 new species. Annals of the Carnegie Museum 80(4): 261–308. doi: 10.2992/007.080.0401

Chatzimanolis S (2013) *Terataki*, a new genus of Staphylinini (Coleoptera: Staphylinidae: Staphylininae) from South America. Zootaxa 3750(3): 251–264. doi: 10.11646/zootaxa.3750.3.5

Chatzimanolis S (2014) Phylogeny of xanthopygine rove beetles (Coleoptera) based on six molecular loci. Systematic Entomology 39(1): 141–149. doi: 10.1111/syen.12040

Clarke DJ (2011) Testing the phylogenetic utility of morphological character systems, with a revision of *Creophilus* Leach (Coleoptera: Staphylinidae). Zoological Journal of the Linnean Society 163: 723–812. doi: 10.1111/j.1096-3642.2011.00725.x

Enderlein G (1912) Die Insekten des Antarkto-Archiplata-Gebietes (Feuerland, Falklands-Inseln, Süd-Georgien). Kungliga svenska Vetenskapsakademiens Handlingar 48(3): 1–170.

Horn W, Kahle I (1935–1937) Über entomologischen Sammlungen Entomologen & Entomo-Museologie (Ein Beitrag zur Geschichte der Entomologie). Entomologische Beihefte aus Berlin-Dahlem 2–4: 1–536.

Kritsky G (1981) Charles Darwin's contribution to entomology, and an index to his insect references. Melsheimer Entomological Series 30: 1–14.

Latreille PA (1802) Histoire naturelle, générale et particulière des Crustacés et des Insectes. Ouvrage faisant suite à l'histoire naturelle générale et particulière, composée par Leclerc de Buffon, et rédigée par C.S. Sonnini, membre de plusieurs sociétés savantes. Tome troisième [3]. Dufart, Paris, xii+467 pp.

Lea AM (1926) On some Australian Coleoptera collected by Charles Darwin during the voyage of the "Beagle". Transactions of the Entomological Society of London 74(2): 279–288. doi: 10.1111/j.1365-2311.1926.tb02239.x

Löbl I (1977) Beitrag zur Kenntnis der Scaphidiidae (Coleoptera) Australiens. Revue Suisse de Zoologie 84(1): 3–69.

Newton AF, Thayer MK (1995) Protopselaphinae new subfamily for *Protopselaphus* new genus from Malaysia, with a phylogenetic analysis and review of the Omaliine Group of Staphylinidae including Pselaphidae (Coleoptera). In: Pakaluk J, Slipinski SA (Eds) Biology, phylogeny, and classification of Coleoptera: papers celebrating the 80th birthday of Roy A. Crowson. Volume 1. Muzeum i Instytut Zoologii PAN, Warsaw, Poland, 219–320.

Scheerpeltz O (1936) Die von Prof. Dr. H. Eidmann gelegentlich seiner im Jahre 1933 nach Brasilien unternommenen Studienreise aufgesammelten Staphyliniden. I. Die in den Nestern von *Atta sexdens* L. aufgefundenen Staphyliniden, nebst einigen Bemerkungen über die Gattung *Scariphaeus* Er. Archiv für Naturgeschichte (N.F.) 5: 483–540.

Sharp D (1884) Staphylinidae. In: Biologia Centrali-Americana. Insecta. Coleoptera 1(2). Taylor & Francis, London, 313–392.

Smith KGV (1987) Darwin's Insects, Charles Darwin's Entomological Notes. Bulletin of the British Museum (Natural History) 14(1): 1–143.

Steel WO (1964) Insects of Campell Island. Coleoptera: Staphylinidae. Pacific Insects monograph 7: 340–375.

Stephens SL (1827–1845) Illustrations of British Entomology, London, 11 vols.

Wasmann E (1902) Riesige Kurzflügler als Hymenopteren-Gäste. (132 Beitrag zur Kenntniss der Myrmekiphilen und Termitophilen.). Insektenborse 19: 267–268, 275–276, 282.

Waterhouse FH (1879) Descriptions of new Coleoptera of geographical interest, collected by Charles Darwin, Esq. Journal of the Linnean Society of London. Zoology. 14: 530–534.

Wheeler QD, Platnick NI (2000) The phylogenetic species concept (sensu Wheeler and Platnick). In: Wheeler QD, Meier R (Eds) Species Concepts and Phylogenetic Theory: A Debate. Columbia University Press, New York, NY, 55–69.

Wollaston TV (1877) Coleoptera Sanctae-Helenae. London, 256 pp.

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RESEARCH ARTICLE

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A new *Hermeuptychia* (Lepidoptera, Nymphalidae, Satyrinae) is sympatric and synchronic with *H. sosybius* in southeast US coastal plains, while another new *Hermeuptychia* species – not *hermes* – inhabits south Texas and northeast Mexico

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Abstract

Hermeuptychia intricata Grishin, **sp. n.** is described from the Brazos Bend State Park in Texas, United States, where it flies synchronously with *Hermeuptychia sosybius* (Fabricius, 1793). The two species differ strongly in both male and female genitalia and exhibit 3.5% difference in the COI barcode sequence of mitochondrial DNA. Setting such significant genitalic and genotypic differences aside, we were not able to find reliable wing pattern characters to tell a difference between the two species. This superficial similarity may explain why *H. intricata*, only distantly related to *H. sosybius*, has remained unnoticed until now, despite being widely distributed in the coastal plains from South Carolina to Texas, USA (and possibly to Costa Rica). Obscuring the presence of a cryptic species even further, wing patterns are variable in both butterflies and ventral eyespots vary from large to almost absent. To avoid confusion with the new species, **neotype** for *Papilio sosybius* Fabricius, 1793, a common butterfly that occurs across northeast US, is designated from Savannah, Georgia, USA. It secures the universally accepted traditional usage of this name. Furthermore, we find that DNA barcodes of *Hermeuptychia* specimens from the US, even those from extreme south Texas, are at least 4% different from those of *H. hermes* (Fabricius, 1775)—type local-

ity Brazil: Rio de Janeiro—and suggest that the name *H. hermes* should not be used for USA populations, but rather reserved for the South American species. This conclusion is further supported by comparison of male genitalia. However, facies, genitalia and 2.1% different DNA barcodes set *Hermeuptychia* populations in the lower Rio Grande Valley of Texas apart from *H. sosybius*. These southern populations, also found in northeastern Mexico, are described here as *Hermeuptychia hermybius* Grishin, **sp. n.** (type locality Texas: Cameron County). While being phylogenetically closer to *H. sosybius* than to any other *Hermeuptychia* species, *H. hermybius* can usually be recognized by wing patterns, such as the size of eyespots and the shape of brown lines on hindwing. "Intricate Satyr" and "South Texas Satyr" are proposed as the English names for *H. intricata* and *H. hermybius*, respectively.

Keywords

Biodiversity, cryptic species, DNA barcodes, neotropical, satyr, *Hermeuptychia gisella*, *Hermeuptychia cucullina*, *Hermeuptychia sosybius kappeli*, female genitalia

Introduction

What could be more exciting than a discovery of a new butterfly species? Perhaps the discovery of a butterfly species in the US that was long overlooked, completely unexpected, and has closest named relatives far away in Bolivia and Brazil. These finds may not be easy to come by, because most of such species are cryptic and appear superficially similar to their more common and well-known relatives. However, DNA-based techniques introduced in taxonomy during the last few decades offer viable tools to facilitate discovery of cryptic species (Bickford et al. 2007).

The genus *Hermeuptychia* was proposed by Forster (1964) on the basis of male genitalia to circumscribe a group of close relatives hardly separable by highly variable wing patterns, but distinct in male genitalia. Lamas (2004) recognized eight named species of *Hermeuptychia* and suggested the existence of several unnamed species in Colombia and Peru. A recent comparative study of DNA barcodes and morphology of male genitalia from all parts of *Hermeuptychia* range revealed congruence between classifications by barcodes and genitalia, hypothesized that *H. gisella* (Hayward, 1957) is a species distinct from *H. cucullina* (Weymer, 1911), and discussed several unnamed species in Brazil (Seraphim et al. 2014). Interestingly, specimens with barcodes and genitalia similar to *H. hermes* (Fabricius, 1775)—type locality Brazil: Rio de Janeiro—were not found north of Costa Rica. Most importantly for this work, Seraphim et al. (2014) outlined several distinct molecular and morphological groups of species, assigned existing names to these groups, illustrated their genitalia and listed genitalia characters in their Table 1. All *Hermeuptychia* specimens from the US (North Carolina, Tennessee and Florida) used by Seraphim et al. (2014) possessed similar DNA barcode sequences and were assigned to morphogroup 4 by male genitalia.

Here, we show that two distinct species from two different morphogroups as defined by Seraphim et al. (2014) fly together at the same location in Texas on the same day. These two species possess very different genitalia in both sexes and 3.5% difference in DNA barcodes. One of these species has traditionally been called *H. sosybius*

(Fabricius, 1793) and the neotype for it is designated herein. The second species is apparently new, and is from the same molecular group with South American species *H. cucullina* (from Peru and Bolivia) and *H. gisella* (from Bolivia and Brazil). This new species is described, discussed and illustrated. Furthermore, we find that DNA barcodes of *Hermeuptychia* from the lower Rio Grande Valley region of Texas (Webb, Zapata, Starr, Hidalgo, and Cameron Counties) form a tight cluster and differ by at least 2% from the barcodes of over 50 *H. sosybius* specimens (divergence average 0.09%, standard deviation 0.19%, maximum below 1%) across its range from North Carolina to Texas (south to Uvalde, Comal, Guadalupe and Brazoria Counties). In addition to DNA barcodes, these south Texas *Hermeuptychia* populations differ from *H. sosybius* by wing patterns and male genitalia (subtly, but quantifiably) and are described here as another new species, bringing the total count of USA *Hermeuptychia* species to three.

Materials and methods

Specimens used in this study were collected in the field under the permit #08-02Rev from Texas Parks and Wildlife Department to NVG, and inspected in the following collections: Texas A&M University Insect Collection, College Station, TX (TAMU); National Museum of Natural History, Smithsonian Institution, Washington, DC (USNM); Natural History Museum, London, UK (BMNH). Standard entomological techniques were used for dissection (Robbins 1991), i.e. abdomen was broken off, soaked for 40 minutes (or until ready) in 10% KOH at 60 °C (or overnight at room temperature), dissected, and subsequently stored in a small glycerol-filled vial on the pin under the specimen. Genitalia and wing venation terminology follows Steinhauser (1981). Length measurements are in metric units and were made from photographs of specimens taken with a scale and magnified on a computer screen. Photographs of immature stages were taken by NVG using Minolta Maxxum 500si 35mm SLR film camera through a 90 mm f/2.8 Tamron SP AF Macro lens (for smaller objects additionally with a Phoenix C/D7 AF 2X Teleconverter) on Kodachrome 25 or Fuji Velvia 50 slide films and slides were scanned using Nikon Super CoolScan 5000 ED film scanner. Photographs of specimens were taken with a Nikon D800 camera through a 105 mm f/2.8G AF-S VR Micro-Nikkor lens; dissected genitalia were photographed in glycerol with a Nikon D200 camera without a lens and through microscopes at 4×–5× magnification. Images were assembled and edited in Photoshop CS5.1. Genitalic photographs were taken in several focus slices and stacked in Photoshop to increase depth of field.

Two legs (cut with scissors into tiny pieces in lysis buffer) of freshly collected specimens, or two legs that were removed from freshly collected specimens and preserved in alcohol for several years, or an abdomen (dropped into lysis buffer as a whole, and after overnight incubation at 56 °C transferred into 10% KOH for genitalia dissection) of older specimens were used to extract genomic DNA with QIAGEN DNeasy blood and tissue kit complemented with EconoSpin columns from Epoch, or Macherey-

Nagel (MN) NucleoSpin® tissue kit following the manufacturer's protocol. Genomic DNA was eluted in a total volume of 120-150 µl QIAGEN AE buffer (concentration of DNA as measured by Promega QuantiFluor® dsDNA System was from 0.01 to 2.5 ng/µl for legs and from 0.005 to 30 ng/µl for abdomens, depending on specimen age and storage conditions) and was stored at -20 °C.

PCR was performed using Invitrogen AmpliTaq Gold 360 master mix in a 20 µl total volume containing less than 10 ng of template DNA and 0.5 µM of each primer. For legs from freshly collected specimens or those preserved in alcohol, the following primers were used to obtain the complete barcode: LepF: 5'-TGTAAAACGACGGCCAGTATTCAACCAATCATAAAGATATTGG-3'and LepR: 5'-CAGGAAACAGCTATGACCTAAACTTCTGGATGTCCAAAAAATCA-3'. For older specimens the following pairs of primers were used: sCOIF (forward, 5'-ATTCAACCAATCATAAAGATATTGG-3') – smCOIR (reverse, 5'-CCTGTTCCAGCTCCATTTTC-3') and bat-smCOIF (forward, 5'-GCTTTTCCTCGTATAAATAATA-3') – sCOIR (reverse, 5'-TAAACTTCTGGATGTCCAAAAAATCA-3'), to amplify barcode in two overlapping segments (307, 408 bp).

The barcodes of the *H. sosybius* neotype (designated below) and *H. hermes kappeli* Anken, 1993 holotype were amplified in four overlapping segments with the following four pairs of *Hermeuptychia*-specific primers: styr-COIF (forward, 5'-CAACCAATCATAAAGATATTGGAAC-3') – styr-bCOIR (reverse, 5'-AAAATTATAATAAAAGCATGRGCTGT-3'), styr-bCOIF (forward, 5'-YCCAGGATTTTTAATTGGAGATG-3') – styr-mCOIR (reverse, 5'-CCTGTYCCACTTCCATTTTCTAC-3'), styr-mCOIF (forward, 5'-TTTTGATTATTACCYCCATCTTT-3') – styr-eCOIR (reverse, 5'-TTCCTACAGCTCAAATAAATAAAGG-3'), and styr-eCOIF (forward, 5'-TTCATTTAGCTGGAATTTCWTCAA-3') – sCOIR (reverse, 5'-TAAACTTCTGGATGTCCAAAAAATCA-3').

For very old specimens (e.g., from 1898 to 1944), amplification of longer DNA segments failed. To obtain their sequences for identification, we developed *Hermeuptychia*-specific primers for very short, about 100 bp fragments, which we call ID tags. Two regions, in which the three USA *Hermeuptychia* species differ from each other the most, were selected and the following primers were designed: styr-ID1F (forward, 5'-TTGAGCAGGAATAATTGGWACAT-3') – styr-ID1R (reverse, 5'-AAAAGCATGRGCTGTAACAA-3') and styr-ID2F (forward, 5'-TTGGAGGATTTGGTAATTGACTT-3') – styr-ID2R (reverse, 5'-AAAGATGGRGGTAATAATCAAAAT-3') to amplify 75 and 56 bp sequence from the specimen (together with both primers, the actual products are 118 and 103 bp).

These primers yielded clear DNA sequence traces (Fig. 65) for 11 out of 12 specimens. The failed traces from DNA voucher 13386A05 showed signs of contamination (i.e., multiple peaks at many positions, probably not even a *Hermeuptychia* sequence) and were inconclusive. Genitalia, however, offered unambiguous identification of this specimen. We did not pursue re-extraction of DNA from the 13386A05 specimen and were satisfied with higher than 90% success rate (11 out of 12) of this method. The oldest specimens from 1898 and likely prior to 1896 (date not specified on the

label of the second specimen, and 1896 is the date of collection donation) yielded excellent traces (e.g., Fig. 65). 6, 1 and 4 specimens of each of the three species were sequenced. For DNA extraction and PCR reactions, they were intermixed and ordered not by species, but as they were placed in USNM collection by curators who did not suspect the presence of more than one species (i.e. semi-randomly, according to DNA voucher numbers assigned to them). Because cross-contamination frequently happens between adjacent specimens, this arrangement alleviates biasing DNA conclusions on the basis of our genitalia and wing pattern-based identification. I.e., if adjacent specimens are the same species (and thus are likely to possess the same DNA barcode), it is more difficult to detect cross-contamination from neighbors. However, if they are different species, disagreement between genitalia-based identification and DNA-based identification would raise suspicions of cross-contamination. All 11 successful DNA identifications were invariably the same as identifications on the basis of genitalia and wing patterns (the voucher 15609E04, Fig. 44, lacked abdomen), and agreed with geographic distribution of these species.

PCR reaction was cleaned up by enzymatic digestion for the whole barcode amplifications of DNA from freshly collected or alcohol preserved specimens and ID tag amplification of old specimens with 4 µl Shrimp Alkaline Phosphatase (20 U/µl) and 1 ul Exonuclease I (1 U/µl) from New England Biolabs. For older specimens that are barcoded in multiple segments, due to the frequent presence of primer dimers and other short non-specific PCR products, Agencourt Ampure XP beads or Invitrogen E-Gel® EX Agarose Gels (followed by Zymo gel DNA recovery kit) were used to select the DNA products of expected length. Sequences were obtained using the M13 primers (for amplification from LepF and LepR primers): 5'-TGTAAAACGACGGCCAGT-3'or 5'-CAGGAAACAGCTATGACC-3'or with primers used in PCR. For the ID tags, PCR products were sequenced in both directions. Sanger sequencing was performed with Applied Biosystems Big Dye Terminator 3.1 kit on ABI capillary instrument in the DNA Sequencing Core Facility of the McDermott Center at UT Southwestern. The resulting sequence traces were proofread in FinchTV <http://www.geospiza.com/Products/finchtv.shtml>. We obtained complete or partial DNA barcode sequences from 85 *Hermeuptychia* specimens. Sequences and accompanying specimen data were submitted to GenBank and received accession numbers KJ025523–KJ025607. Data about these specimens are provided in Table 1.

Additional DNA sequences were downloaded from GenBank <http://genbank.gov/> using accession numbers provided in Seraphim et al. (2014) or were found by BLAST <http://blast.ncbi.nlm.nih.gov/> searches using sequences obtained by us to query "nr/nt" database. Information about specimens with sequences used in this study is in Table 1. All sequences were aligned manually since they matched throughout their length without insertions or deletions, and analyzed using the Phylogeny.fr server at <http://www.phylogeny.fr/> with default parameters (Dereeper et al. 2008), namely, Kimura 2-parameters model (Kimura 1980) was used to compute evolutionary distances from aligned DNA sequences and BioNJ (Gascuel 1997) algorithm was used to build trees.

Table 1. Data for specimens with DNA sequences used in this study.

Species	Voucher	GenBank	Locality	Date	Collector
H. sosybius	NVG-696	KJ025523	OK: Atoka Co., 13 air mi E of Atoka, 34.41186 -95.91044, 225 m	29-Aug-2009	Nick V. Grishin
H. sosybius	NVG-1632	KJ025524	TX: Lamar Co., 11.5 air mi NW of Paris, FM1499 @ Sanders Cr., 140 m	25-Apr-1998	Nick V. Grishin
H. sosybius	NVG-1630	KJ025525	TX: Marion Co., nr. Carter L., 50 m	28-Sep-1996	Nick V. Grishin
H. sosybius	NVG-1633	KJ025526	TX: Marion Co., nr. Carter L., 50 m	29-Sep-1996	Nick V. Grishin
H. sosybius	NVG-1606	KJ025527	TX: Wise Co., LBJ National Grassland, 300 m	3-Aug-1998	Nick V. Grishin
H. sosybius	NVG-783	KJ025528	TX: Tyler Co., John H. Kirby SF, 40 m	19-Mar-2011	Nick V. Grishin
H. sosybius	NVG-784	KJ025529	TX: Tyler Co., John H. Kirby SF, 40 m	19-Mar-2011	Nick V. Grishin
H. sosybius	NVG-785	KJ025530	TX: Tyler Co., John H. Kirby SF, 40 m	19-Mar-2011	Nick V. Grishin
H. sosybius	NVG-786	KJ025531	TX: Tyler Co., John H. Kirby SF, 40 m	19-Mar-2011	Nick V. Grishin
H. sosybius	NVG-1537	KJ025532	TX: Fort Bend Co., Brazos Bend SP, Horseshoe L. tr., 29.38193 -95.61141, 15 m	17-Aug-2013	Nick V. Grishin
H. sosybius	NVG-1538	KJ025533	TX: Fort Bend Co., Brazos Bend SP, Horseshoe L. tr., 29.38193 -95.61141, 15 m	17-Aug-2013	Nick V. Grishin
H. sosybius	NVG-1539	KJ025534	TX: Fort Bend Co., Brazos Bend SP, Horseshoe L. tr., 29.38193 -95.61141, 15 m	17-Aug-2013	Nick V. Grishin
H. sosybius	NVG-1540	KJ025535	TX: Fort Bend Co., Brazos Bend SP, Horseshoe L. tr., 29.38193 -95.61141, 15 m	17-Aug-2013	Nick V. Grishin
H. sosybius	NVG-1542	KJ025536	TX: Fort Bend Co., Brazos Bend SP, Horseshoe L. tr., 29.38193 -95.61141, 15 m	17-Aug-2013	Nick V. Grishin
H. sosybius	NVG-1543	KJ025537	TX: Fort Bend Co., Brazos Bend SP, Horseshoe L. tr., 29.38193 -95.61141, 15 m	17-Aug-2013	Nick V. Grishin
H. sosybius	NVG-1544	KJ025538	TX: Fort Bend Co., Brazos Bend SP, Horseshoe L. tr., 29.38193 -95.61141, 15 m	17-Aug-2013	Nick V. Grishin
H. sosybius	NVG-1545	KJ025539	TX: Fort Bend Co., Brazos Bend SP, Horseshoe L. tr., 29.38193 -95.61141, 15 m	17-Aug-2013	Nick V. Grishin
H. sosybius	NVG-1546	KJ025540	TX: Fort Bend Co., Brazos Bend SP, Horseshoe L. tr., 29.38193 -95.61141, 15 m	17-Aug-2013	Nick V. Grishin
H. sosybius	NVG-1547	KJ025541	TX: Fort Bend Co., Brazos Bend SP, Horseshoe L. tr., 29.38193 -95.61141, 15 m	17-Aug-2013	Nick V. Grishin
H. sosybius	NVG-1549	KJ025542	TX: Fort Bend Co., Brazos Bend SP, Horseshoe L. tr., 29.38193 -95.61141, 15 m	17-Aug-2013	Nick V. Grishin
H. sosybius	NVG-1550	KJ025543	TX: Fort Bend Co., Brazos Bend SP, Horseshoe L. tr., 29.38193 -95.61141, 15 m	17-Aug-2013	Nick V. Grishin
H. sosybius	NVG-1552	KJ025544	TX: Fort Bend Co., Brazos Bend SP, Horseshoe L. tr., 29.38193 -95.61141, 15 m	17-Aug-2013	Nick V. Grishin
H. sosybius	NVG-1553	KJ025545	TX: Fort Bend Co., Brazos Bend SP, Horseshoe L. tr., 29.38193 -95.61141, 15 m	17-Aug-2013	Nick V. Grishin
H. sosybius	NVG-1557	KJ025546	TX: Fort Bend Co., Brazos Bend SP, nr. Hale L., 29.38008 -95.58473, 16 m	17-Aug-2013	Nick V. Grishin

Species	Voucher	GenBank	Locality	Date	Collector
H. sosybius	NVG-1559	KJ025547	TX: Fort Bend Co., Brazos Bend SP, nr. Hale L., 29.38008 -95.58473, 16 m	17-Aug-2013	Nick V. Grishin
H. sosybius	NVG-1561	KJ025548	TX: Fort Bend Co., Brazos Bend SP, nr. Hale L., 29.38008 -95.58473, 16 m	17-Aug-2013	Nick V. Grishin
H. sosybius	NVG-1562	KJ025549	TX: Fort Bend Co., Brazos Bend SP, nr. Hale L., 29.38008 -95.58473, 16 m	17-Aug-2013	Nick V. Grishin
H. sosybius	NVG-1564	KJ025550	TX: Fort Bend Co., Brazos Bend SP, nr. Hale L., 29.38008 -95.58473, 16 m	17-Aug-2013	Nick V. Grishin
H. sosybius	NVG-1566	KJ025551	TX: Fort Bend Co., Brazos Bend SP, nr. Hale L., 29.38008 -95.58473, 16 m	17-Aug-2013	Nick V. Grishin
H. sosybius	NVG-1567	KJ025552	TX: Fort Bend Co., Brazos Bend SP, nr. Hale L., 29.38008 -95.58473, 16 m	17-Aug-2013	Nick V. Grishin
H. sosybius	13385H04	KJ025553	TX: Comal Co., New Braunfels	3-Oct-1981	
H. sosybius	13385H11	KJ025554	TX: Williamson Co., Florence	3-Sep-1974	J. Parkinson
H. sosybius	13385H03	KJ025555	TX: Uvalde Co., Utopia	10-Jun-1992	D. E. Gaskin & EAL
H. sosybius	13385H05	KJ025556	TX: Uvalde Co., Utopia	{9-23}-Sep-1994	D. E. Gaskin & EAL
H. sosybius	13385H06	KJ025557	TX: Uvalde Co., Utopia	{9-23}-Sep-1994	D. E. Gaskin & EAL
H. sosybius	13385H07	KJ025558	TX: Uvalde Co., Utopia	{13-22}-Apr-1995	D. E. Gaskin
H. sosybius	13385H08	KJ025559	TX: Uvalde Co., Utopia	{13-22}-Apr-1995	D. E. Gaskin
H. sosybius	13385G12	KJ025560	FL: Highlands Co., Lake Placid, Archbold Biological Station	17-Feb-1985	D. C. Ferguson
*H. sosybius**	13386A07	KJ025561	GA: Chatham Co., Savannah	28-Jul-1958	Coll. Gordon B. Small
*H. sosybius***	NVG-1845	KJ025562	FL: N of L. Okeechobee	29-Mar-1983	Ralf H. Anken
H. sosybius	15609E04	KJ025563	FL: Pinellas Co., St. Petersburg	**3-Nov-1938**	H. E. Wilford
H. sosybius	13385G10	KJ025564	SC: Clarendon Co.	**Aug-1909**	
H. sosybius	13385H09	KJ025565	TX: Bastrop Co., Bastrop	**prior to 1896**	Collection of O. Meske
H. sosybius	13386A01	KJ025566	TX: Guadalupe Co., Seguin	**26-Oct-1905**	F. C. Pratt
H. sosybius	13386A04	KJ025567	LA: Jackson Parish, Jonesboro	**4-Jun-1920**	G. W. Rawson
H. sosybius	13386A06	KJ025568	LA: Jefferson Parish, Harahan	**11-Aug-1944**	W. D. Field
H. hermybius	NVG-1603	KJ025569	TX: Cameron Co., E of Brownsville	17-Mar-2003	Nick V. Grishin
H. hermybius	NVG-1607	KJ025570	TX: Cameron Co., E of Brownsville	18-Jan-2003	Nick V. Grishin
H. hermybius	NVG-1609	KJ025571	TX: Cameron Co., E of Brownsville	30-Mar-2003	Nick V. Grishin
H. hermybius	NVG-1610	KJ025572	TX: Cameron Co., E of Brownsville	9-Mar-2003	Nick V. Grishin
H. hermybius	NVG-1611	KJ025573	TX: Cameron Co., E of Brownsville	14-Mar-2003	Nick V. Grishin
H. hermybius	NVG-1612	KJ025574	TX: Cameron Co., E of Brownsville	16-Mar-2003	Nick V. Grishin
H. hermybius	NVG-1628	KJ025575	TX: Cameron Co., E of Brownsville	19-Oct-1997	Nick V. Grishin
H. hermybius	NVG-1695	KJ025576	TX: Hidalgo Co., 1.5 air mi SE of Relampago, Rio Rico Rd., 26.07 -97.891, 21 m	19-Oct-2013	William R. Dempwolf
H. hermybius	NVG-1698	KJ025577	TX: Hidalgo Co., 1.5 air mi SE of Relampago, Rio Rico Rd., 26.07 -97.891, 21 m	19-Oct-2013	William R. Dempwolf
H. hermybius	NVG-1699	KJ025578	TX: Hidalgo Co., 1.5 air mi SE of Relampago, Rio Rico Rd., 26.07 -97.891, 21 m	19-Oct-2013	William R. Dempwolf
H. hermybius	NVG-1712	KJ025579	TX: Starr Co., Rio Grande City, Fort Ringgold, 26.3707 -98.8064, 45 m	20-Oct-2013	William R. Dempwolf
H. hermybius	NVG-1714	KJ025580	TX: Starr Co., Rio Grande City, Fort Ringgold, 26.3707 -98.8064, 45 m	20-Oct-2013	William R. Dempwolf

Species	Voucher	GenBank	Locality	Date	Collector
H. hermybius	NVG-1726	KJ025581	TX: Starr Co., Roma, S of Roma International Bridge, 26.4035 -99.0175, 50 m	20-Oct-2013	William R. Dempwolf
H. hermybius	NVG-1727	KJ025582	TX: Starr Co., Roma, S of Roma International Bridge, 26.4035 -99.0175, 50 m	20-Oct-2013	William R. Dempwolf
H. hermybius	NVG-1735	KJ025583	TX: Starr Co., 0.5 mi S of Fronton, 26.399 -99.085, 50 m	20-Oct-2013	William R. Dempwolf
H. hermybius	NVG-1737	KJ025584	TX: Starr Co., 0.5 mi S of Fronton, 26.399 -99.085, 50 m	20-Oct-2013	William R. Dempwolf
H. hermybius	NVG-1747	KJ025585	TX: Starr Co., Salineno @ Rio Grande, 26.51463 -99.11633, 53 m	23-Oct-2013	William R. Dempwolf
H. hermybius	NVG-1635	KJ025586	TX: Zapata Co., San Ygnacio @ Rio Grande, 92 m	7-Oct-2007	Nick V. Grishin
H. hermybius	13385H10	KJ025587	TX: Webb Co., Laredo	15-Apr-1949	E. L. Todd
H. intricata	NVG-1541	KJ025588	TX: Fort Bend Co., Brazos Bend SP, Horseshoe L. tr., 29.38193 -95.61141, 15 m	17-Aug-2013	Nick V. Grishin
H. intricata	NVG-1548	KJ025589	TX: Fort Bend Co., Brazos Bend SP, Horseshoe L. tr., 29.38193 -95.61141, 15 m	17-Aug-2013	Nick V. Grishin
H. intricata	NVG-1551	KJ025590	TX: Fort Bend Co., Brazos Bend SP, Horseshoe L. tr., 29.38193 -95.61141, 15 m	17-Aug-2013	Nick V. Grishin
H. intricata	NVG-1554	KJ025591	TX: Fort Bend Co., Brazos Bend SP, nr. Hale L., 29.38008 -95.58473, 16 m	17-Aug-2013	Nick V. Grishin
H. intricata	NVG-1555	KJ025592	TX: Fort Bend Co., Brazos Bend SP, nr. Hale L., 29.38008 -95.58473, 16 m	17-Aug-2013	Nick V. Grishin
H. intricata	NVG-1556	KJ025593	TX: Fort Bend Co., Brazos Bend SP, nr. Hale L., 29.38008 -95.58473, 16 m	17-Aug-2013	Nick V. Grishin
H. intricata	NVG-1558	KJ025594	TX: Fort Bend Co., Brazos Bend SP, nr. Hale L., 29.38008 -95.58473, 16 m	17-Aug-2013	Nick V. Grishin
*H. intricata**	NVG-1560	KJ025595	TX: Fort Bend Co., Brazos Bend SP, nr. Hale L., 29.38008 -95.58473, 16 m	17-Aug-2013	Nick V. Grishin
H. intricata	NVG-1563	KJ025596	TX: Fort Bend Co., Brazos Bend SP, nr. Hale L., 29.38008 -95.58473, 16 m	17-Aug-2013	Nick V. Grishin
H. intricata	NVG-1565	KJ025597	TX: Fort Bend Co., Brazos Bend SP, nr. Hale L., 29.38008 -95.58473, 16 m	17-Aug-2013	Nick V. Grishin
H. intricata	NVG-1629	KJ025598	TX: San Jacinto Co., Sam Houston NF, USF217 @ Big Creek, 58 m	12-Apr-1998	Nick V. Grishin
H. intricata	NVG-1631	KJ025599	TX: Brazoria Co., Bar-X Ranch, Rd. 971N, 29.13252 -95.58340, 7 m	4-Mar-2000	Nick V. Grishin
H. intricata	13385G07	KJ025600	SC: Charleston Co., McClellanville, Wedge Plantation	6-Apr-1970	D. C. Ferguson
H. intricata	13385H01	KJ025601	FL: Alachua Co., Gainesville	12-Mar-1983	Scott W. Gross
H. intricata	13385H02	KJ025602	FL: "Putnam Co | Shell Bluff Landing"	29-Sep-1985	George Balogh
H. intricata	13386A03	KJ025603	LA: Jefferson Parish, Harahan	28-Jun-1944	W. D. Field
H. intricata	13385G08	KJ025604	SC: Clarendon Co.	**9-Aug-1898**	
H. intricata	13385G09	KJ025605	SC: Clarendon Co.	**Aug-1910**	
H. intricata	13385G11	KJ025606	SC: Clarendon Co.	**Aug-1910**	
H. intricata	13386A02	KJ025607	"Flatbush LI"	**prior to 1941**	G. P. Engelhardt Coll.
H. sosybius	DNA-ATBI-0799	GU089906*	NC: Swain Co., AN9, Smokemont Stables, 35.5504 -83.3084	20-Jul-2004	R. M. Pyle
H. sosybius	NSHer-EUA07	KF466083*	TN: Rutheford Co., 35.70 -86.33	2009	A. V. Z. Brower
H. sosybius	NSHer-EUA08	KF466084*	TN: Rutheford Co., 35.70 -86.33	2009	A. V. Z. Brower

Species	Voucher	GenBank	Locality	Date	Collector
H. sosybius	DNA-ATBI-0847	GU089907*	TN: Blount Co. AN2, Cades Cove, along Forge Cr. Rd. 35.583 -83.838	20-Jul-2004	R. M. Pyle
H. sosybius	DNA-ATBI-0848	GU089908*	TN: Blount Co. AN2, Cades Cove, along Forge Cr. Rd. 35.583 -83.838	20-Jul-2004	R. M. Pyle
H. sosybius	DNA-ATBI-0849	GU089909*	TN: Blount Co. AN2, Cades Cove, along Forge Cr. Rd. 35.583 -83.838	20-Jul-2004	R. M. Pyle
H. sosybius	DNA-ATBI-4110	GU088393*	TN: Sevier Co., Lyon Spring Rd., 35.6 -83.4	22-May-2005	Segebarth
H. sosybius	DNA-ATBI-4109	GU088394*	TN: Sevier Co., Lyon Spring Rd., 35.6 -83.4	22-May-2005	Segebarth
H. sosybius	NSHer-EUA02	KF466080*	FL: Gainesville, 29.65 -82.32	Apr-2009	K. R. Willmott
H. sosybius	NSHer-EUA03	KF466081*	FL: Gainesville, 29.65 -82.32	Apr-2009	K. R. Willmott
H. sosybius	NSHer-EUA06	KF466082*	FL: Gainesville, 29.65 -82.32	Apr-2009	K. R. Willmott
H. cucullina	NSHer-PE03	KF466142*	Peru		C Peña
H. gisella	NSHer-J29	KF466092*	Brazil: São Paulo, Serra do Japí, Jundiaí, -23.22 -46.92	26-Feb-2008	P. E. C. Peixoto
H. atalanta	R10_CA_SP	JN109040*	Brazil: São Paulo, Ribeirão Cachoeira, Campinas		
H. hermes	NSHer-MG08	KF466108*	Brazil: Minas Gerais, Serra do Cipó, Jaboticatubas, -18.20 -43.50	Dec-2005	A. R. M. Silva
H. maimoune	NSHer-CO04	KF466021*	Colombia, Meta, Bosque Bavaria, 4.18 -73.65	8-Oct-2006	M. A. Marín
H. pimpla	CP04-10	GU205843*	Peru: Quebrada Siete Jeringas		
H. harmonia	CP06-93	GU205842*	Peru: Quebrada Siete Jeringas		
H. fallax	NSHer-J17	KF466089*	Brazil: São Paulo, Serra do Japí, Jundiaí, -23.22 -46.92	26-Feb-2008	P. E. C. Peixoto
Megisto cymela	DNA-ATBI-4114	GU088434*	TN: Sevier Co., Lyon Spring Rd., 35.6 -83.4	22-May-2005	Segebarth
H. intricata ?	DNA96-016	AY508548*	Costa Rica: Puntarenas Province		

Abbreviations: SP State Park; L. Lake; Cr Creek tr. trail; nr. near; Co. County; NF National Forest; SF State Forest Rd. Road

* after the species name indicates primary type specimen, ** is *Hermeuptychia hermes kappeli* holotype

* after the GenBank number indicates that it was retrieved from GenBank, all other sequences were determined by us in this study

Only DNA ID tags were obtained for the oldest specimens and their dates are shown in **bold font.**

Results and discussion

Taxonomic status of various *Hermeuptychia* populations in Texas has been puzzling (Miller and Brown 1981, Pelham 2008). Some authors treated them as conspecific with eastern USA populations, either under the name *H. sosybius* (Opler and Malikul 1992, Allen 1997, Glassberg et al. 2000, Opler and Warren 2002, Glassberg 2007) or *H. hermes* (Howe 1975, Opler and Krizek 1984, Scott 1986, Neck 1996). Others apparently assigned more southern populations to *H. hermes*, reserving the name *H. sosybius* for eastern butterflies (Miller and Brown 1981, brief comment in Neck 1996, Pelham 2008, Warren et al. 2013).

As a part of a barcoding exercise to shed some light on taxonomy of *Hermeuptychia*, we obtained DNA sequences from several samples across Texas. The results were not as expected. In fact, populations from extreme south Texas with the small

eyespots phenotype characteristic of *H. hermes* revealed barcodes more similar to those across eastern US. Genitalic examinations showed that even specimens from Tamaulipas and San Luis Potosí, Mexico possessed characters of morphogroup 4 (i.e. the one that includes *H. sosybius*) from Seraphim et al. (2014).

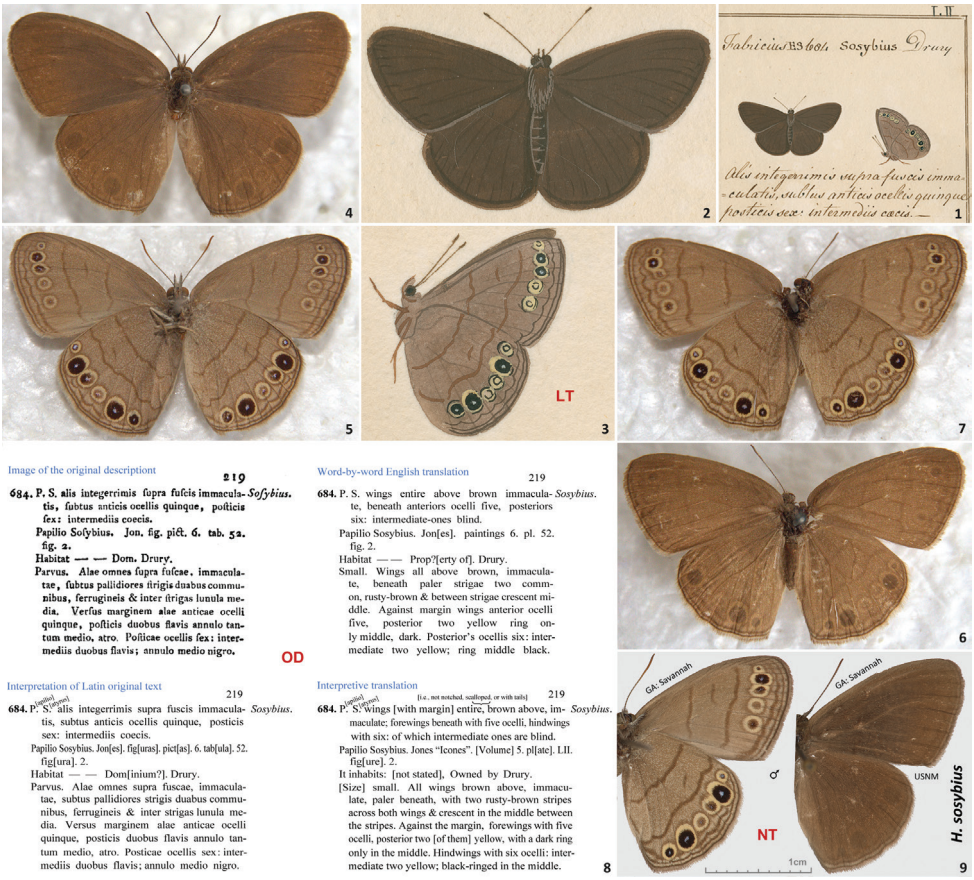
However, much to our surprise, several specimens from southeast (but not southernmost) Texas, namely from the Brazos Bend State Park in Fort Bend County near Houston, possessed barcodes 3.5% different from those of all other USA populations and, as found by BLAST (Altschul et al. 1990), more than 2% different from all other available sequences (except one, discussed below) in GenBank (Benson et al. 2013). Both males and females were in the sample with the unusual barcodes.

Suspecting DNA introgression, similar to that reported by Zakharov et al. (2009), or some yet unexplained irregularities with barcodes, we critically inspected genitalia of these butterflies. Even more surprisingly, both male and female genitalia of the specimens with unusual barcodes differed profoundly from those with classic morphogroup 4 (suggested *H. sosybius*) barcodes, and male genitalia were more similar to morphogroups 5, 6 and possibly 7 of Seraphim et al. (2014), differing in certain details from all of them. The morphogroups 5 and 6 included specimens from Peru and south Brazil and were associated with the names *H. cucullina* (Weymer, 1911) (type locality: Bolivia) and *H. gisella* (Hayward, 1957), *reinstated status* (type locality: Bolivia) per data provided by Seraphim et al. (2014). Morphogroup 7 referred to an unnamed phenotype from South Brazil.

Apparently, in Fort Bend County, Texas there exist two sympatric and synchronic *Hermeuptychia* species (collected on the same day at exactly the same spot!), one from morphogroup 4 and the other one more similar to morphogroups 5, 6 & 7. Interestingly, a possible closest named relative of this second species is either *H. gisella* or *H. cucullina*, documented from Bolivia and central to southeastern Brazil. The situation might be analogous to another butterfly recently described from the US, *Strymon solitario* Grishin & Durden, 2012, whose possible sibling is *Strymon jacqueline* Nicolay & Robbins, 2005 from Peru (Grishin and Durden 2012).

Historical investigations into *Papilio sosybius* Fabricius, 1793

The two *Hermeuptychia* species from east Texas are markedly different in genitalia of both sexes and in DNA barcodes. However, upon close inspection of wing patterns, we failed to find strong diagnostic differences that would hold against individual variation. Searching for additional specimens revealed the presence of both species across the eastern US from Texas to Florida and South Carolina, but didn't reveal obvious wing pattern differences either. This posed a problem with the taxonomic identity of these two species, as it was uncertain which one, if any, is *H. sosybius* described by Fabricius (1793: 219). In his brief description, Fabricius referenced unpublished drawings ("Icones") by William Jones (Vane-Wright 2010): "Jon. fig. pict. 6. tab. 52. fig. 2." and Drury specimens, but did not state the locality these specimens came from (Figs



Figures 1–9. Historical illustrations and specimens of *H. sosybius*, its original description, and neotype. **1–3** Illustration of *H. sosybius* syntype(s) by William Jones [1745–1818] from an unpublished book called the "Icones" (Vane-Wright 2010), currently in Oxford University Museum of Natural History, UK (Smith 1986). **1** shows the upper right quadrant of the plate LII from Volume 5 **2, 3** are magnified cropped images off this plate showing dorsal and ventral aspects, respectively; ventral image (**3**) is rotated clockwise for the ease of comparison with specimens. The specimen with ventral side illustrated (**3** and on the right in **1**) is designated as the lectotype herein and is apparently lost **4–7** Two possible syntypes of *H. sosybius* from the Macleay collection (Macleay Museum, The University of Sydney, Australia). Neither specimen bears any labels **4, 6** show dorsal aspect and **5, 7** show ventral aspect **8** Original description of *H. sosybius* and its translations. Note that the Jones illustrations of *H. sosybius* are currently bound within Volume 5, and not 6 as per description **9** Neotype of *H. sosybius* (designated herein, also see Figs 10–11, genitalia Fig. 62p, DNA barcode tree Fig. 66b), in USNM collection, from USA: Georgia: Chatham Co., Savannah, 28-Jul-1958, leg. G. B. Small, genitalia NVG131102-61, DNA voucher 13386A07, GenBank accession for mitochondrial DNA COI barcode KJ025561. Scale bar refers to **9** only, other images are scaled approximately. Images **1–3** are copyright of Oxford University Museum of Natural History, UK (used with permission), and images **4–7** are copyright of Macleay Museum, The University of Sydney, Australia and are photographed by Robert Blackburn (used with permission).

1–3, 8). The specimens used by Jones to sketch from and those in the Drury collection (the same specimens?) are *H. sosybius* syntypes. With the help of Kathleen Santry (Head of Archival Collections), we obtained high resolution digital images of the *H. sosybius* drawing by Jones from the Hope Library, Oxford University Museum of Natural History (Oxford, UK). The images show a dorsal side of a specimen on the left, which is uniformly dark-brown; and a ventral side of a specimen on the right (Figs 1–3). Consistent with the Fabricius description (Fig. 8), the ventral surface of wings is paler-brown, with darker brown submedial, postmedial, sinuous submarginal and marginal lines across both wings and end-of-cell dark-brown dash on each wing. Submarginal eyespots are large (compared to *H. hermes*): 5 on the forewing, the 2nd and 3rd from the costa are larger, black-ringed and pupiled; 6 on the hindwing, the 2nd, 5th and 6th from the costa are larger, black-ringed and pupilled, 2nd and 5th being the largest. Generally, this wing pattern is consistent with both *Hermeuptychia* species from southeast Texas.

We have taken the following steps to trace the type specimens of *H. sosybius*. First, we studied relevant publications. For instance, Zimsen (1964: 561) specifies for "Papilio Sosybius": "». . . Dom. Drury« – " with no specimen location mention after the dash. In contrast, for "Papilio Hermes", Zimsen (1964: 514) lists "»in Brasilia Mus. Banks«, ... – London 1 specimen." Indeed, there is presently a specimen presumed to be *H. hermes* type in Banks collection in BMNH (see images in Warren et al. 2013). Miller and Brown (1981: 191) state "Type lost, a Drury specimen." Pelham (2008: 404) echoes: "Type(s) probably lost."

Second, we consulted knowledgeable historians and scholars of Lepidoptera. John V. Calhoun kindly provided the following information: Drury's collection was sold at auctions and the catalogs of sales did not list specimens of *H. sosybius*. However, species names for many sold specimens were not given. It is possible that the types of *H. sosybius* were acquired by Macleay and are in the Macleay Museum (Sydney, Australia). However, even if *H. sosybius* specimens could be found in the Macleay collection, it will be nearly impossible to figure out which (if any) served as types. Gerardo Lamas (pers. comm.) was not able to trace *H. sosybius* syntypes in his comprehensive search for the primary type specimens of all Neotropical butterflies, and expressed an opinion that it would be very difficult to support the status of any found specimens as syntypes.

Nevertheless, as a third step, we contacted the Macleay Museum staff with a request to search for specimens similar to those illustrated by Jones in the Macleay collection. After extensive search of the Macleay holdings (housed in two places), Robert Blackburn, armed with the Jones illustrations and photographs of *H. sosybius* specimens, was able to find four *Hermeuptychia* specimens of potential interest. According to Mr. Blackburn (pers. comm.), "the history of these 4 is hard [to determine] due to the absolute lack of labels. Much of the material in these drawers came from a mixture of sources, between William Sharp Macleay's trading network of entomologists and Alexander Macleay's purchases at auctions. I think that butterflies like these would be most likely to be Alexander Macleay purchases, and probably came through the purchase of Dru Drury's collections at auction. I think it's absolutely possible that they are 1780's specimens, maybe even through John Abbot, as many of the other butterflies

in these drawers are labelled 'Georgia'." Two of these (Figs 4–7) would be identifiable as *H. sosybius* by facies. Unfortunately, neither specimen bears any labels and it will be very difficult to find supporting evidence that these are indeed syntypes. Even if these specimens are from the Drury collection, since Drury exchanged material, it is impossible to know that these are the original specimens, or the ones acquired after the *H. sosybius* description.

Next, we compared these specimens with the Jones illustrations. The wing pattern and shape of the specimen with abdomen intact (female, Figs 6–7) do not agree closely with the Jones illustrations (Figs 1–3). Most notably, Jones's illustration of the ventral aspect (Fig. 3) shows two forewing eyespots with strongly developed black rings (near the apex, 2nd and 3rd from the costa), and the specimen has only one (2nd from the costa, the 3rd eyespot entirely lacks black and is more similar to the two posterior eyespots, Fig. 7). The postmedial dark line on ventral hindwing is shaped differently. e.g., it is directed basad near costa in the illustration and is directed distad in the specimen. Other differences in details of placement and shape of eyespots and dark lines are equally obvious, and it is not likely that this specimen was the model for the Jones illustration.

The specimen lacking the abdomen (Figs 4–5) is more similar to the specimen(s) illustrated by Jones, i.e. both 2nd and 3rd eyespots on the forewing are black-ringed and the postmedial hindwing line (slightly) bends basad at costa. However, it seems to be mounted differently than the Jones's dorsal image shows, i.e. the hindwings that are lowered on the Jones image and touch each other with inner margins, are widely apart in the specimen (Fig. 4). Ventral patterns (in case Jones image Fig. 3 depicts a different specimen from that shown on dorsal image Fig. 2) also differ in detail. In particular, the 3rd hindwing eyespot from the costa lacks black and is more similar to the 4th from costa eyespot in the illustration, but is clearly black-ringed and larger than the 4th eyespot in the specimen (Fig. 5). The submedial and postmedial dark lines on both wings are farther apart in the illustration than in the specimen. The postmedial dark line is strongly bent, directed basad and reaches the hindwing inner margin at an angle in the illustration (more similar to the specimen illustrated in Fig. 47), but is almost perpendicular to the inner margin near the tornus in the specimen. In our opinion, it is not very likely that these obvious pattern differences are caused by inaccuracy of the Jones illustration, in part because we see *Hermeuptychia* specimens (e.g. Fig. 47) that are more similar in such patterns to the Jones illustration than the specimen in Fig. 5. We see that *Hermeuptychia* specimens with the characters illustrated by Jones exist, and it seems more likely that their characters were illustrated, rather that invented by Jones. Therefore, we conclude that neither of the specimens from the Macleay collection is the one illustrated by Jones. John V. Calhoun who has vast experience dealing with the analysis of historical illustrations agrees with this opinion (pers. comm.).

To stabilize nomenclature, similarly to Calhoun (2006), we designate the specimen with ventral aspect illustrated by Jones in Volume 5, plate LII (second species illustrated on this plate), topmost image on the right (reproduced here as Fig. 3) in his unpublished manuscript known as "Icones" (Vane-Wright 2010) and referred to as "Jon. fig.

pict. 6. tab. 52. fig. 2." by Fabricius (1793) in his original description (Fig. 8) as the lectotype of *Papilio sosybius* Fabricius, 1793. It is possible that the Jones illustration may be a composite, amalgamated image of several specimens. If that was the case, the lectotype is the specimen that contributed the most to the illustration. I.e., of all specimens used as models for this possibly composite illustration, the largest number of characters depicted are from the lectotype. As discussed above, our search for this specimen was unsuccessful, and the lectotype is most likely lost. Because we were not able to find definitive wing pattern characters to differentiate between the two eastern US *Hermeuptychia* species (one of which is *H. sosybius* and the other one is not), and the Fabricius description (1793, Fig. 8) augmented with Jones illustration of the lectotype (Fig. 3) is generally consistent with both species, we proceeded with the neotype designation.

Neotype designation for *Papilio sosybius* Fabricius, 1793

We believe that there is an exceptional need for the neotype to clarify the taxonomic identity of *H. sosybius* and to define which one of the two USA *Hermeuptychia* species this name refers to. We hypothesize that it is more likely that the species from morphogroup 4 – i.e. *H. sosybius* as defined by Seraphim et al. (2014: Table 1 to list its male genitalia characters), characters detailed below – that is widely distributed across eastern US and is more common in collections, is the species that Fabricius named "Papilio Sosybius". For instance, inspection of *Hermeuptychia* holdings in the USNM collection from 13 US states across its distribution range (MD, VA, SC, GA, TN, AR, AL, KY, MS, LA, TX & FL) revealed that one species outnumbered the other one more than 20 to 1 (169 vs. 8 specimens). The characters seen in specimens of this entity that is significantly more prevalent in collections are consistent with the original description of *H. sosybius* and Jones illustration of the lectotype. Most importantly, the Jones ventral drawing (Fig. 3) shows: 1) a rather straight postmedial brown line on the forewing towards the costa; 2) postmedial brown line on hindwing bulges basad near the costa and 3) it bulges distad somewhat anterior or at the level of the vein M_3 (should be between large and small eyespots in typical specimens of the more common species, and between two middle small eyespots, closer to the posterior small eyespot, in the rarely collected species). These three characters (indicated in Fig. 68, first image from the left below the line, voucher NVG-1542) are typical of morphogroup 4 specimens. However, the third character (the bulge anterior or posterior of vein M_3) is somewhat inconclusive from the Jones drawing (Fig. 3) and could possibly be interpreted either way, creating uncertainty with the lectotype identification from the Jones illustration.

In most specimens of eastern US species from a different morphogroup (5, 6, or 7), the forewing postmedial brown line bends basad from vein M_1 towards the costa, the hindwing postmedial brown line is more straight near the costa, and it bulges distad around vein M_3 (between the two small eyespots in the middle, closer to the posterior eyespot). While the sample of 21 specimens is too small to evaluate the reliability of the wing pattern characters and even this sample already shows variation in

these characters (e.g. in some specimens the forewing line is straight towards the costa), morphogroup 4 species seems to be more consistent with Jones's lectotype drawing in patterns. Combining this albeit rather weak wing pattern evidence with the 20 to 1 ratio of morphogroup 4 specimens found in collections, its possibly wider distribution across eastern US, and the usage of the name "sosybius" in publications to denote this phenotype and DNA barcode (e.g. Seraphim et al. 2014), we conclude that morphogroup 4 species better represents *H. sosybius* of Fabricius, and look for a neotype specimen of this species.

While this species cannot be confidently identified by wing patterns at the moment, it can be differentiated from other *Hermeuptychia* species by the following combination of male genitalia characters (Figs 60a, d, g, j, 61c, 62o–z2): (1) comparatively large, more gracile and weaker sclerotized (paler) genital capsule (Fig. 60a); (2) medially wider uncus with more prominently convex sides in dorsal (or ventral) view, uncus appears truncated at the apex in dorsal (or ventral) view, but the width of uncus at the apex is generally less than 2/3 of the width of uncus at the narrowest point near the base (Figs 60a, d, 61c); (3) uncus dorsally flatter towards the apex, but convex in lateral view towards the base and with a prominent, thin, membranous carina in basal half (Fig. 60j); (4) valvae elongated, with a saccular lobe, cucullus more gracile, narrower and longer, it projects for close to half of its length farther than the distal end of gnathos (lateral view, Fig. 60g, j); (5) cucullus narrow at the apex, usually with three to five (mostly four) prominent apical teeth (Fig. 60g, j); (6) interior surface of cucullus ventrally without a prominent bulge, best seen in ventral view (Fig. 60d); (7) aedeagus is more gracile, narrower and longer, especially near the distal end, evenly curved or bent distad the middle (Fig. 60d, g, j); (8) longer than wide phallobase (Fig. 60g, j); (9) larger and wider saccus, but shorter than 2/3 of valva length (Fig. 60d). Further analyses and comparisons of genitalia characters between *Hermeuptychia* species are given in Table 1 of Seraphim et al. (2014). In addition, specimens from morphogroup 4 of Seraphim et al. (2014) clustered as molecular group G in the DNA barcode tree. All 40 DNA barcodes we obtained for specimens of the species that we are selecting to represent *H. sosybius*, closely clustered together with the sequences of group G in our trees as well (Fig. 66b).

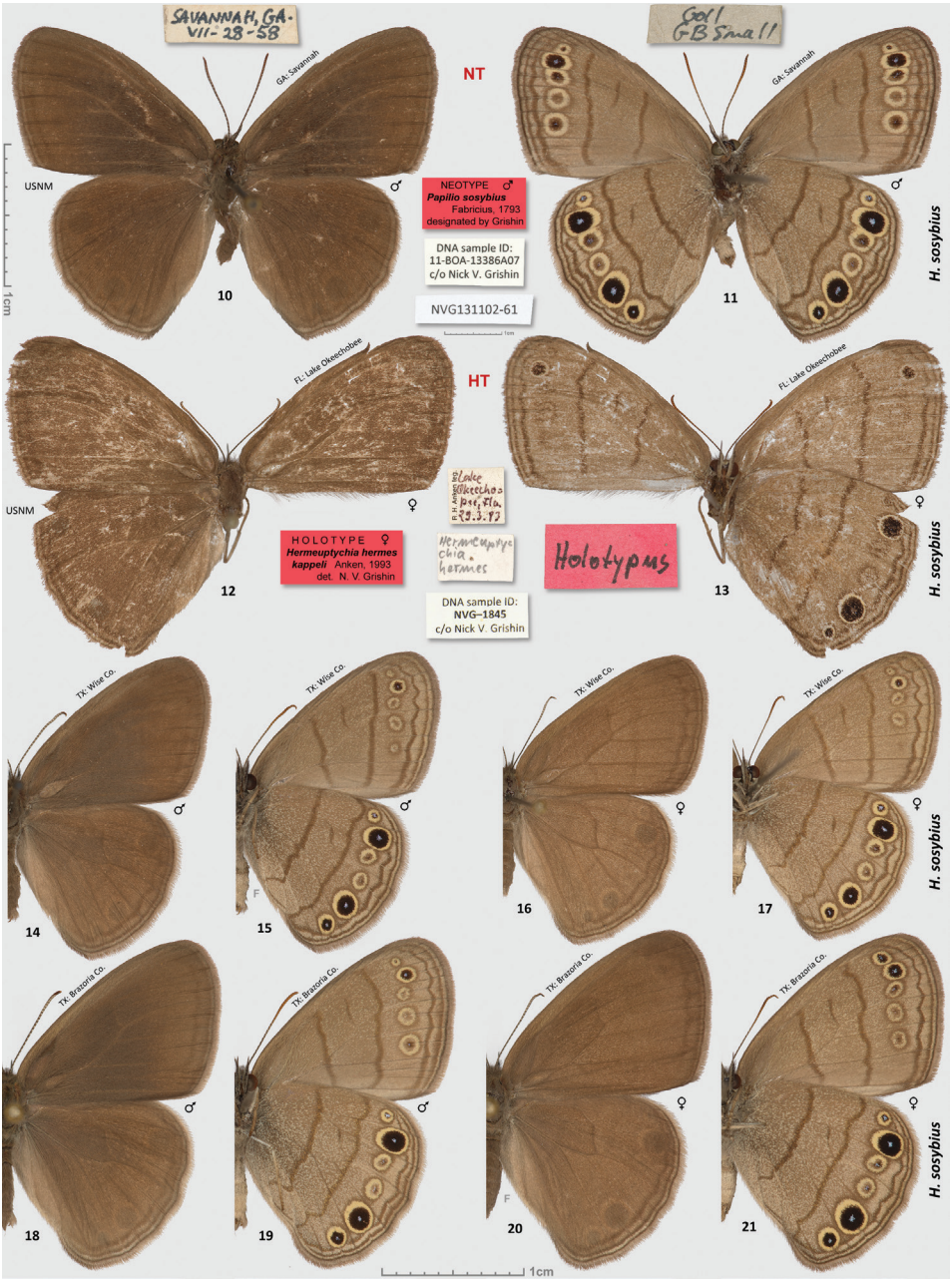
From the Jones drawing, it is not possible to unambiguously determine the sex of the illustrated specimens because *Hermeuptychia* are not prominently dimorphic sexually, although the darker color of the specimen shown in dorsal view and wing shape on both illustrations is more consistent with a male. We decided to choose a male specimen as the neotype because male genitalia have been used more widely in *Hermeuptychia* taxonomy, were illustrated for the majority of known species by Forster (1964) and extensively analyzed by Seraphim et al. (2014).

The locality of *H. sosybius* types was not stated in the original description and currently remains unknown. However, we could attempt to deduce it by comparative analysis of wing patterns on Jones's drawings. Large eyespots on both wings, some mostly black and pupilled with pale blue are distinctive. Because the size of eyespots is highly variable in *Hermeuptychia*, it is conceivable that the Drury's specimens origi-

nated in Central or even South America. However, due to very strong development of eyespots and characteristic shape of rusty-brown lines ventrally on both wings, Jones's drawings are more likely to depict eastern USA *Hermeuptychia*. Most importantly, the name "sosybius" has been applied to these USA populations historically, and in the interest of stability it is best to secure this name for these populations. If the *H. sosybius* types were collected in the USA, it is most likely that Drury obtained them from John Abbot and they originated in the eastern coastal US, possibly in Georgia or Virginia (John V. Calhoun, pers. comm.). Populations of the morphogroup 4 species are continuous and widely distributed in east US (Opler et al. 2013), and they show essentially identical DNA barcode sequences from North Carolina to south Texas (Fig. 66b). Genitalia of inspected specimen do not reveal notable differences across the range either. Recently, Robbins and Lamas (2006) designated a neotype of *Calycopis cecrops* (Fabricius, 1793), a species described by Fabricius in the same publication with *H. sosybius* and under similar circumstances (i.e., Jones illustrations) from "Indiis", later proposed to be "one of the states along the eastern coast of the United States between Virginia and Georgia, and probably the latter" by Field (1967). Robbins and Lamas (2006) have chosen the neotype to be from USA: Georgia: Chatham Co., Savannah. We could not have done better, and simply follow their example.

A male specimen (Figs 9–11, genitalia Fig. 62p) bearing three rectangular labels: yellowing white, handprinted on one side - || SAVANNAH, GA. | VII-28-58 ||, grayish, handwritten on the other side - || Coll | G B Small ||; white printed - || DNA sample ID: | 11-BOA-13386A07 | c/o Nick V. Grishin ||; white printed - || NVG131102-61 ||; and a plastic glycerin-filled vial with genitalia on the same pin with the specimen, is hereby designated as the neotype of *Papilio sosybius* Fabricius, 1793. Upon this publication, red printed label || NEOTYPE ♂ | *Papilio sosybius* | Fabricius, 1793 | designated by Grishin || will be added. Forewing length of the neotype is 15.5 mm, and this specimen can be recognized by a unique pattern of minor damage to scale cover on wings above, i.e. a longitudinal scratch in the distal half of the left forewing discal cell and a scratch across the discal area of both right wings (Fig. 10). Prior to genitalia dissection, abdomen of the neotype was used to extract total genomic DNA as described in Materials and methods section. The neotype wing pattern mostly agrees with the original description and is similar to Jones illustrations, and the choice of the species is consistent with the usage of this name. The original type locality is not specified in the description (Fig. 8), and the new type locality of *H. sosybius* according to ICZN Article 76.3 (ICZN 1999) is USA: Georgia: Chatham Co., Savannah. The neotype is in the National Museum of Natural History, Smithsonian Institution, Washington, DC (USNM). It is our pleasure to select this excellent specimen collected by Gordon B. Small, one of the most knowledgeable and finest collectors of American, and in particular Panamanian, butterflies (Nicolay 1989), who "knew more about butterflies than any person" (DeVries 1989) and whose exquisite and comprehensive collection of over 50,000 masterfully prepared specimens, rich in rare and undescribed species, is in USNM for future generations to study.

Barcode sequence of the neotype: Genbank accession KJ025561, 658 base pairs:



Figures 10–21. *Hermeuptychia sosybius.* **10**–**11** neotype designated herein and **12**–**13** holotype of *H. hermes kappeli*, data in text **14**–**15** ♂ USA: Texas, Wise Co., LBJ National Grassland, ex ovum, eclosed 3-Aug-1998, leg. N. V. Grishin **16**–**17** ♀ ibid, 10-Aug-1998 **18**–**19** ♂ USA: Texas, Brazoria Co., Bar-X Ranch, Rd. 971N, ex ovum, eclosed 18-Apr-2000, leg. N. V. Grishin **20**–**21** ♀ ibid, 21-Apr-2000. Dorsal/ventral surfaces are in even/odd-numbered figures. Labels are shown for primary types in-line with the specimens and are reduced 2.5-fold compared to specimens as indicated by a smaller scale bar. "F" specifies mirror image (left-right inverted).

AACTTTATATTTTATTTTTGGTATTTGAGCAGGAATAATTGGAACAT-
CATTAAGTTTAATTATCCGAATAGAATTAGGTAACCCAGGATTTT-
TAATTGGAGATGACCAAATTTATAATACTATTGTTACAGCTCATGCTTT-
TATTATAATTTTTTTTTATAGTAATACCTATTATAATTGGAGGATTTG-
GTAATTGACTTATTCCTTTAATATTAGGAGCTCCTGATATAGCTTTTC-
CGCGTATAAATAATATAAGATTTTGATTATTACCTCCATCTTTAATTTT-
ATTAATTTCTAGCAGTATTGTAGAAAATGGAAGTGGAACAGGATGAACT-
GTTTACCCCCCTCTTTCATCTAATATTGCTCATAGAGGTTCTTCAGTA-
GATTTAGCAATTTTTTCTCTTCATTTAGCTGGAATTTCATCAATTTTAG-
GAGCTATTAATTTTATTACAACAATTATTAATATACGAATTAATAATA-
TATCTTATGATCAAATACCTTTATTTATTTGAGCTGTAGGAATTACT-
GCTCTTCTTTTACTTCTCTCATTACCTGTTTTAGCAGGAGCTATTAC-
CATACTTCTTACTGATCGAAATTTAAATACATCATTTTTTGATCCT-
GCAGGAGGAGGAGATCCTATTTTATATCAACATTTATTT

We believe that our designation of the neotype completely satisfies qualifying conditions of the ICZN Article 75.3 (ICZN 1999). I.e., the exceptional need for the neotype arose due to our discovery that more than one *Hermeuptychia* species was present in eastern USA, and neither the original description, nor the only available illustration of *Papilio sosybius* Fabricius, 1793 lectotype was sufficient to determine which species, if any, was *H. sosybius*. The neotype was designated to clarify the taxonomic identity of *H. sosybius*, i.e., to define which one of the two eastern US *Hermeuptychia* species (than cannot be confidently told apart by the wing patterns) was *H. sosybius*, and to clarify the type locality of *H. sosybius*, which was not stated in the original description (Art. 75.3.1). *H. sosybius* was differentiated from other *Hermeuptychia* species by its DNA barcode given above that placed it in a molecular group G of *Hermeuptychia* species per Seraphim et al. (2014), and by its attribution to the morphogroup 4 by Seraphim et al. (2014), who listed its diagnostic male genitalia characters (Seraphim et al. 2014: Table 1); these characters were elaborated upon and illustrated in this study, e.g., Fig. 60a, d, g, j (Art. 75.3.2). The neotype specimen could be recognized by its labels and appearance as described above and was illustrated in Figs 9–11 (Art. 75.3.3). The reasons to believe that the *H. sosybius* lectotype was lost and the steps we took to trace it were detailed above under the heading "Historical investigations into *Papilio sosybius* Fabricius, 1793" (Art. 75.3.4). We presented the evidence that the neotype was consistent with prior knowledge about *H. sosybius* and was in full agreement with the traditional and current usage of this name (Art. 75.3.5). The neotype specimen came from the general geographic area of hypothesized origin of the lectotype (Art. 75.3.6). Finally, we stated that the neotype is in USNM collection (Art. 75.3.7).

The name "*Hermeuptychia hermes kappeli*" suggested by Anken (1993), type locality "Lake Okeechobee (Nord), Florida, U.S.A." was regarded as a junior subjective synonym of *H. sosybius* by Calhoun (1997), Lamas (2004) and Pelham (2008). The *H. h. kappeli* holotype (Table 1, Figs 12–13, USA: Florida: N of Lake Okeechobee, 29-Mar-1983, leg. R. H. Anken, to be deposited in USNM) kindly mailed to us by Dr. R. H. Anken lacks the abdomen, rendering genitalic examination impossible. We ob-

tained a barcode sequence from its legs (Genbank accession KJ025562) to compare *H. h. kappeli* with other *Hermeuptychia*. The sequence was 100% identical with that of *H. sosybius* neotype (Fig. 66b) and was more than 3.4% different from either the new species, or *H. hermes*. Wing patterns (see discussion below) of the *H. h. kappeli* holotype were also more consistent with *H. sosybius* than with the new species to be described below. Therefore, *H. h. kappeli* is either a subspecies of *H. sosybius*, or its subjective junior synonym as previously proposed (Calhoun 1997, Lamas 2004, Pelham 2008). Because DNA barcodes may not vary with subspecies, and we did not study sufficient material from near the type localities of both taxa, we cannot comment on the validity of *H. s. kappeli* as a subspecies and adopt the latest treatment (Pelham 2008). However, several butterflies in Florida tend to be regarded as distinct subspecies from nominal taxa with type localities in Georgia or South Carolina (Pelham 2008, Warren et al. 2013). Therefore, a more detailed comparative analysis of wing patterns in *H. sosybius* populations might be desirable.

No other names have been proposed for North and Central American *Hermeuptychia*. Now, after the clarification of the morphogroup 4 species identity by the *H. sosybius* neotype designation and conclusion that *H. h. kappeli* is either a subspecies or synonym of *H. sosybius*, we can proceed with the description of a different morphogroup (5, 6, or 7) species from southeast Texas.

***Hermeuptychia intricata* Grishin, sp. n.**
http://zoobank.org/A89BD0A9-9CE9-4DC7-9EFD-42F77A34B2DD
http://species-id.net/wiki/Hermeuptychia_intricata
Figs 22–35, 40–43, 60c, f, i, l, 61a, 62n, 64i–p, 65 part, 66 part, 67 part, 68 part

Description. Male (n=14, Figs 22–23, 28–29, 32, 34–35, 40–43, 68 part) – holotype forewing length = 16.5 mm. Forewing triangular, rounded at apex and tornus, costal and outer margins convex, inner margin almost straight, mildly concave mediad, two discal cell veins bulged at bases, vein 2A thickened basad. Hindwing rounded, almost circular. Wings dorsally dark-brown with sparse olive-beige overscaling and two darker-brown terminal lines. Wings ventrally pale-brown, paler towards inner margin of forewing, with extensive beige overscaling, particularly along veins in distal part in some specimens; submedial and postmedial dark-brown lines and dark-brown end-of-cell streak (smaller on hindwing) between them; forewing postmedial line bent basad near costa in many specimens; hindwing postmedial line almost straight near costa, rarely convex basad and typically convex distad posterior of M_3 (between the two small eyespots in the middle, closer to posterior eyespot); two terminal dark-brown evenly curved marginal lines, dark-brown sinuous submarginal line, and row of submarginal eyespots basad of the sinuous line and posteriad of outer discal line, largest eyespots black-centered and pupiled with pale-blue scales: on forewing, largest eyespot in cell M_1-M_2, eyespot in cell R_5-M_1 black-centered in some specimens; on hindwing, largest eyespots in cells Cu_1-Cu_2 and M_1-M_2, a smaller one in cell Cu_2-1A+2A, even smaller, but still black-centered

Figures 22–31. *Hermeuptychia intricata.* **22**–**23** holotype, others are paratypes, data in text and Table 1. Sexes and DNA voucher codes are: **24** ♀ NVG-1554 **25** ♀ NVG-1565 **26**–**27** ♀ 13385G09 **28**–**29** ♂ NVG-1631 **30**–**31** ♀ NVG-1629. Dorsal/ventral surfaces are in even/odd-numbered figures, except **24**, which is ventral. Labels are shown for the holotype and are reduced 2.5-fold compared to specimens as indicated by a smaller scale bar. "F" specifies mirror image (left-right inverted).

and pale-blue pupilled in cell Rs-M_1, and two smallest, usually without black, but in some specimens pale-blue pupilled eyespots in cells M_2-M_3 and M_3-Cu_1. Fringes monochrome, a little paler than the ground color of wings. Head, palpi, thorax and abdomen dark-brown above, paler and mostly beige beneath. Antennae dark-brown above with pale scales at segments, orange-brown at the club, beneath beige basad, orange-brown in distal half. Legs brown with beige scales. Male genitalia (n=14: 12 dissected, 2 inspected *in situ*, Figs 60c, f, i, l, 61a, 62n) – typical for the genus, smaller and darker in color (more sclerotized) than those of *H. sosybius*. Tegumen dome-like, rounded at margins. Uncus leaf-shaped in dorsal view, angled to the sides, roof-like, convex distally but almost flat basally in lateral view, without thin, membranous carina in basal half; apex of uncus pointed, not truncated. Gnathos arms thin, wide apart, divergent, about the same length as uncus. Valvae narrow, elongated with thin cuculli extending past gnathos not farther than a third of their length; cucullus more rounded at apex, usually with a couple of small teeth; cucullus ventrally with inner medial bulge. Saccus about the same length as cucullus, narrow. Aedeagus elongated, almost straight, only slightly and evenly curved, not bent, broader and shorter compared to *H. sosybius*, with a smaller, about as long as wide phallobase. Female (n=8, Figs 23–27, 30–31, 33, 68 part) – similar to male in facies, with slightly more rounded wings and dorsally paler in color. Female genitalia (n=8, Fig. 64i–p) with antrum darker in color and smaller than that of *H. sosybius*. Ostium bursae ellipsoidal, its ventral margin longer than dorsal margin. Antrum narrower anteriad, almost triangular in ventral view, somewhat kidney-shaped in lateral view, mostly symmetric. Ductus and corpus bursae each in length similar to antrum; corpus bursae with two signa, spines in a signum broad, leaf-shaped, usually shingled in two rows.

Barcode sequence of the holotype. Genbank accession KJ025595, 658 base pairs:
AACTTTATATTTTATTTTTGGTATTTGAGCAGGAATAATTGGTA-
CATCATTAAGTTTAATTATCCGAATAGAATTAGGTAATCCAGGATTTT-
TAATTGGAGATGACCAAATTTATAATACTATTGTTACAGCTCATGCTTT-
TATTATAATTTTTTTTTATAGTAATACCCATTATAATTGGAGGATTTGG-
TAATTGACTTGTCCCTTTAATATTAGGAGCTCCTGATATAGCTTTCC-
CACGTATAAATAATATAAGATTTTGATTATTACCCCCATCTTTAATTTT-
ATTAATTTCTAGTAGTATTGTAGAAAATGGAAGTGGGACAGGATGAACA-
GTTTACCCCCCCCTCTCATCTAATATTGCTCATAGAGGTTCTTCAGTA-
GATTTAACAATTTTTTCACTTCATTTAGCTGGAATTTCTTCAATCTTAG-
GAGCTATTAATTTTATTACAACAATTATTAACATACGAATCAATAATA-
TATCTTATGATCAAATACCTTTATTTATTTGAGCTGTAGGAATTACA-
GCTCTTCTTTTACTTCTTTCATTACCTGTTTTAGCAGGAGCTATTAC-
TATACTTCTTACTGATCGAAATTTAAATACATCATTTTTTGATCCT-
GCAGGAGGAGGAGATCCTATTTTATATCAACATTTATTT

In addition to the holotype, barcodes and ID tags were obtained for 19 paratypes (15 full-length barcodes and 4 ID tags, see Table 1, GenBank accessions: KJ025588–KJ025607, except KJ025595, which is the holotype). Full length barcodes revealed five haplotypes differing from each other by just 1 to 3 base pairs (less than 0.5%).

Figures 32–47. *H. intricata* paratypes and *H. sosybius* specimens. **32–35, 40–43** *H. intricata* **36–39, 44–47** *H. sosybius*; data in text and Table 1. Sexes and DNA voucher codes are: **32** ♂ 13385G11 **33** ♀ 13385G08 **34** ♂ 13385G07 **35** ♂ 13386A02 **36** ♂ 13385G10 **37** ♀ 13385G12 **38** ♀ 13386A04 **39** ♀ 13386A06 **40** ♂ 13385H02 **41** ♂ 13385H01 **42** ♂ 13386A05 **43** ♂ 13386A03 **44** ♂ 15609E04 **45** ♀ 13385H07 **46** ♂ 13385H08 **47** ♂ 13386A01. All specimens are in USNM collection. Ventral wing surfaces are shown. "F" specifies mirror image (left-right inverted).

The haplotype of the holotype was more frequently observed (Fig. 66b) and other four haplotypes were confined to a single specimen in the sample.

Type material. Holotype: ♂, has the following four rectangular labels: white printed - || USA: TEXAS: Fort Bend Co. | Brazos Bend State Park, | Hale Lake, 29.3801°–95.5847°| 17-Aug-2013 Grishin N.V. ||; white printed - || DNA extraction | NVG-1560 | 2013-09-05 ||; white printed - || Genitalia vial # | NVG130927-14 | Prep. N. V. Grishin ||; red printed - || HOLOTYPE ♂ | *Hermeuptychia* | *intricata* Grishin ||. The holotype is illustrated in Figs 22–23, 60c, f, i, l, & 68 (first image), and the Genbank accession for its DNA COI barcode sequence is KJ025595. Upon publication, the holotype will be deposited in the National Museum of Natural History, Smithsonian Institution, Washington, DC (USNM). **Paratypes:** 13 ♂♂ and 8 ♀♀, all from USA. Of these, 2 ♂♂ and 5 ♀♀ with the same data as the holotype; and 3 ♂♂ (DNA vouchers: NVG-1541, NVG-1548, & NVG-1551) from 2.5 km to the east, i.e. USA: Texas: Fort Bend Co., Brazos Bend State Park, Horseshoe Lake trail, latitude 29°22'54.96", longitude –95°36'41.06", elevation 15 m, 17-Aug-2013, leg. N. V. Grishin. Sexes and GenBank accessions|DNA voucher numbers|genitalia codes (na if not available) for these paratypes (the same format is used below for others) are: ♂ KJ025588|NVG-1541|NVG131003-03, ♂ KJ025589|NVG-1548|na, ♂ KJ025590|NVG-1551|na, ♀ KJ025591|NVG-1554|NVG130927-07, ♂ KJ025592|NVG-1555|NVG131003-04, ♂ KJ025593|NVG-1556|NVG131003-05, ♀ KJ025594|NVG-1558|NVG130927-08, ♀ KJ025596|NVG-1563|NVG130927-11, ♀ KJ025597|NVG-1565|NVG130927-12, ♀ na|na|NVG131003-10. All but one of these paratypes are illustrated in Figs 24, 25, 68 (above the line). 1 ♂ Texas: Brazoria Co., Bar-X Ranch, Rd. 971N, 29.13252 -95.58340, 7 m, 4-Mar-2000, leg. Nick V. Grishin, KJ025599|NVG-1631|NVG131017-08 (Figs 28–29, 62n). 1 ♀ Texas: San Jacinto Co., Sam Houston National Forest, USF217 @ Big Creek, 58 m, 12-Apr-1998, leg. Nick V. Grishin, KJ025598|NVG-1629|NVG131017-06 (Figs 30–31). 1 ♂ South Carolina: Charleston Co., McClellanville, Wedge Plantation, 6-Apr-1970, leg. D. C. Ferguson, KJ025600 | 13385G07|NVG131102-38 (Fig. 34). 1 ♀ South Carolina: Clarendon Co., 9-Aug-1898, KJ025604|13385G08|NVG131102-39 (Fig. 33). 1 ♀ ibid., Aug-1910, KJ025605|13385G09|NVG131102-40 (Figs 26–27). 1 ♂ ibid., Aug-1910, KJ025606|13385G11|NVG131102-42 (Fig. 32). 1 ♂ Florida: "Putnam Co | Shell Bluff Landing", 29-Sep-1985, George Balogh, KJ025602|13385H02|NVG131102-45 (Fig. 40). 1 ♂ Florida: Alachua Co., Gainesville, 12-Mar-1983, leg. Scott W. Gross, KJ025601|13385H01|NVG131102-44 (Fig. 41). 1 ♂ Louisiana: Jefferson Parish, Harahan, 28-Jun-1944, W. D. Field, KJ025603|13386A03|NVG131102-57 (Fig. 43). 1 ♂ Louisiana: Jackson Parish, Jonesboro, na|13386A05|NVG131102-59 (Fig. 42). 1 ♂ "Flatbush LI" (specimen curated in the USNM among *Hermeuptychia* from Louisiana), collected prior to 1941, G. P. Engelhardt Coll., KJ025607|13386A02|NVG131102-56 (Fig. 35).

Type locality. USA: Texas: Fort Bend Co., Brazos Bend State Park, near Hale Lake, latitude 29°22'48.27", longitude –95°35'05.02", elevation 16 m. This locality is

by a wooded, partly open, lowland hiking trail (near and along the park paved road) from a parking lot towards the Big Creek, north of the Hale Lake.

Etymology. The name refers to the difficulty in recognizing this very distinct species and its intricate ventral wing patterns. The name is an adjective.

Distribution. Generally, this is a species of eastern US coastal plains and is currently documented from Texas, Louisiana, Florida, and South Carolina (Fig. 67). It is expected to be more widely distributed in the region and the exact boundaries of the range remain to be investigated. For instance, photographs of live individuals from Alabama: Bibb Co., Blue Girth Creek, 08-VIII-2004 & 18-VI-2005 by Vitaly Charny (Warren et al. 2013, specimens not collected, excluded from the type series) exhibit characters more consistent with *H. intricata* than with *H. sosybius* (see discussion below). Furthermore, it is difficult to interpret the locality label for the last listed paratype other than "Flatbush Long Island" [New York, Kings Co.]. However, *Hermeuptychia* has not been recorded that far north–northernmost records are from southern New Jersey and southern Pennsylvania (Opler et al. 2013)–therefore this specimen might have been mislabeled. Nevertheless, searches for this species in the coastal New York/New Jersey area might be interesting to probe its northern distribution limits. An additional specimen (not examined, excluded from the type series) from Costa Rica: Puntarenas Province, GenBank accession AY508548 (Murray and Prowell 2005) has DNA sequence with only 1 bp difference (over 435 base pair C-terminal segment of the barcode) from the USA *H. intricata* barcodes. Unless this sequence is a contamination, it is possible that the Costa Rican specimen is *H. intricata*, which may be ranging southwards at least to Costa Rica. It is apparent, however, that *H. intricata* is either more restricted in distribution and local, or significantly less common than *H. sosybius*, because several dozen available barcode sequences of *Hermeuptychia* specimens from different parts of the range in east US (NC, TN, FL, LA, OK and TX, see Fig. 66b) clearly group with *H. sosybius*, and a sample of 177 genitalically inspected *Hermeuptychia* specimens from 13 US states (MD, VA, SC, GA, TN, AR, AL, KY, MS, LA, TX & FL) in the USNM yielded only 8 *H. intricata* (less than 5%). We hope that a timely description of this species within a few months after its initial discovery will stimulate further studies of this interesting cryptic-in-facies butterfly, which, however, can be easily distinguished from its more common congener by genitalia (Figs 60a, c, d, f, g, i, j, l, 61a, c, 62n–z2 & 64a–p) and DNA barcodes (Fig. 66). All known *H. sosybius* records should be scrutinized in search for *H. intricata*.

Diagnosis. In wing pattern, the new species is very similar to *H. sosybius*. We were not able to find solid diagnostic characters for the new species, and only hypothetical field marks could be suggested (see discussion). However, it could be easily identified by many distinctive characters of genitalia.

Males of the new species possess: (1) smaller and more robust and darker genital capsule, even in males with larger body size (Fig. 60c)–genitalia of *H. sosybius* from various parts of the range are larger and look "wider" and are paler (Fig. 60a); (2) narrower and apically pointed uncus (Fig. 60c, f)–uncus of *H. sosybius* is wider and appears truncated at the apex in dorsal or ventral views (Fig. 60a, d); (3) uncus that

is more angled to the sides along the dorsal "rim", thus appearing "higher" in lateral view (Fig. 60l), but flatter basally due to the lack of prominent carina, vs. a dorsally flatter uncus in distal half, with a well-developed thin, membranous carina in basal half in *H. sosybius* (Fig. 60j); (4) shorter and stouter cucullus, which projects for less than a third of its length farther than the distal ends of gnathos arms (lateral view, Fig. 60i, l)–cucullus in *H. sosybius* is more gracile, narrower and longer, it projects for close to half of its length farther than the distal end of gnathos (lateral view, Fig. 60g, j); (5) cucullus more rounded at the apex, usually with a couple of barely defined, very small apical teeth, vs. three to five (mostly four) larger teeth in *H. sosybius*; (6) interior surface of cucullus ventrally with a more prominent bulge, best seen in ventral view (Fig. 60f vs. 60d); (7) more stout, thicker and shorter penis, best seen in ventral view (Fig. 60f)–penis is more gracile, narrower and longer, especially near the distal end, in *H. sosybius* (Fig. 60d); (8) shorter phallobase, which is about as long as wide (Fig. 60i, l), vs. phallobase that is much longer than wide in *H. sosybius* (Fig. 60g, j); (9) smaller and narrower saccus (Fig. 60f), vs. larger and wider one in *H. sosybius* (Fig. 60d); (10) more obtuse angle formed by the tegumen and vinculum in lateral view (Fig. 60l), vs. typically more acute angle in *H. sosybius* (Fig. 60j).

Females of the new species possess: (I) narrower ostium bursae and smaller, darker antrum (Fig. 64i, j)–ostium bursae and antrum are larger and antrum is paler in color in *H. sosybius* (Fig. 64a, b); (II) ventral margin of ostium bursae that extends farther back than its dorsal margin (Fig. 64k, l)–dorsal margin extends posterior of ventral margin in *H. sosybius* (Fig. 64a, b); (III) antrum that is narrower anteriad, almost triangular in ventral view and symmetric (Fig. 64k, m), vs. rounder, cup-like, slightly asymmetric to the left antrum in *H. sosybius* (Fig. 64e); (IV) more bent antrum, kidney-shaped in lateral view (Fig. 64n), than that of *H. sosybius* (Fig. 64j); (V) signa composed of wider, more flattened and rounder spines, mostly in two rows, vs. narrower spines in three to five irregular rows in *H. sosybius*.

Characters (2) and (3) in males (more pointed apex of uncus and uncus more angled to the sides from the central "rim") seem to be the easiest to examine without full dissection by brushing the scales off the abdomen tip, even in dry specimens (Fig. 62a, c). Identification of dry females might be more problematic due to abdomen shriveling, however, in freshly caught individuals, ostium bursae and antrum can be easily exposed by squeezing the abdomen in distal third, and the character (II) becomes observable (relative position of ostium bursae margins). Due to these very significant and easily observed differences in genitalia, identification in the field immediately after capture is expected to be straightforward, however, more work remains to be done to discover diagnostic wing pattern characters.

DNA barcodes, consistently with genitalia, set the new species far apart from sympatric *H. sosybius*, and the difference is about 3.5%, which is significantly higher than "a clear threshold for intra- and interspecific mean distances around 2%", as quoted from the recent comprehensive analysis of *Hermeuptychia* (Seraphim et al. 2014).

While the discovery of this second (and new) *Hermeuptychia* species in eastern USA was very unexpected to us, the next finding is less surprising, although also interest-

ing. Our analysis of DNA barcodes of Texas *Hermeuptychia* revealed that populations from the lower Rio Grande Valley region of Texas (Webb, Zapata, Starr, Hidalgo, and Cameron Counties) form a tight cluster differing by at least 2% from closely clustered barcodes (divergence average 0.09%, standard deviation 0.19%, maximum below 1%) of over 50 *H. sosybius* specimens across its range from North Carolina to Texas (south to Uvalde, Comal, Guadalupe and Brazoria Counties, Figs 66–67). These south Texas (and northeast Mexico) *Hermeuptychia* populations are phenotypically characterized by smaller and more uniformly sized eyespots and more undulated brown lines. This butterfly has been called "*H. hermes*" in some of the recent literature that advocates the presence of two *Hermeuptychia* species in the US (Miller and Brown 1981, brief comment in Neck 1996, Pelham 2008, Warren et al. 2013). However, DNA barcodes clearly and confidently group these populations with *H. sosybius* (Fig. 66a, bootstrap support above 80%, about 2% sequence difference), and *H. hermes* sequences are more than 4% different from either of these [Fig. 66a and Seraphim et al. (2014)]. According to DNA barcodes, *H. hermes* – type locality Brazil: Rio de Janeiro – is in a different species group and clusters with *H. maimoune* (A. Butler, 1870) rather than with *H. sosybius* (Fig. 66a). Analysis of male genitalia agrees with this conclusion. Indeed, genitalia of south Texas specimens are clearly from the morphogroup 4 (i.e. *H. sosybius*) possessing all the characters specified by Seraphim et al. (2014) and are very different from those of *H. hermes* [see Forster (1964) and Seraphim et al. (2014) for illustrations]. Most obviously, *H. hermes* has much longer saccus compared to shorter and more constricted in the middle valvae. Nevertheless, in addition to at least 2% different barcodes, south Texas morphogroup 4 populations differ from eastern *H. sosybius* in facies to the extent that researchers have been treating them as a species distinct from *H. sosybius* (Miller and Brown 1981, Pelham 2008, Warren et al. 2013). Our analysis agrees with this conclusion. Furthermore, we find subtle, but quantifiable, differences in male genitalia between *H. sosybius* and south Texas *Hermeuptychia* populations. Evidence presented above suggests that the name *H. hermes* should not be applied to them. Since currently there are no named species in the *H. sosybius* group [i.e., molecular group G and morphogroup 4 of Seraphim et al. (2014)] other than *H. sosybius*, and south Texas populations fall confidently in the *H. sosybius* group (Fig. 66a), they represent an unnamed species that is described here.

Hermeuptychia hermybius Grishin, sp. n.

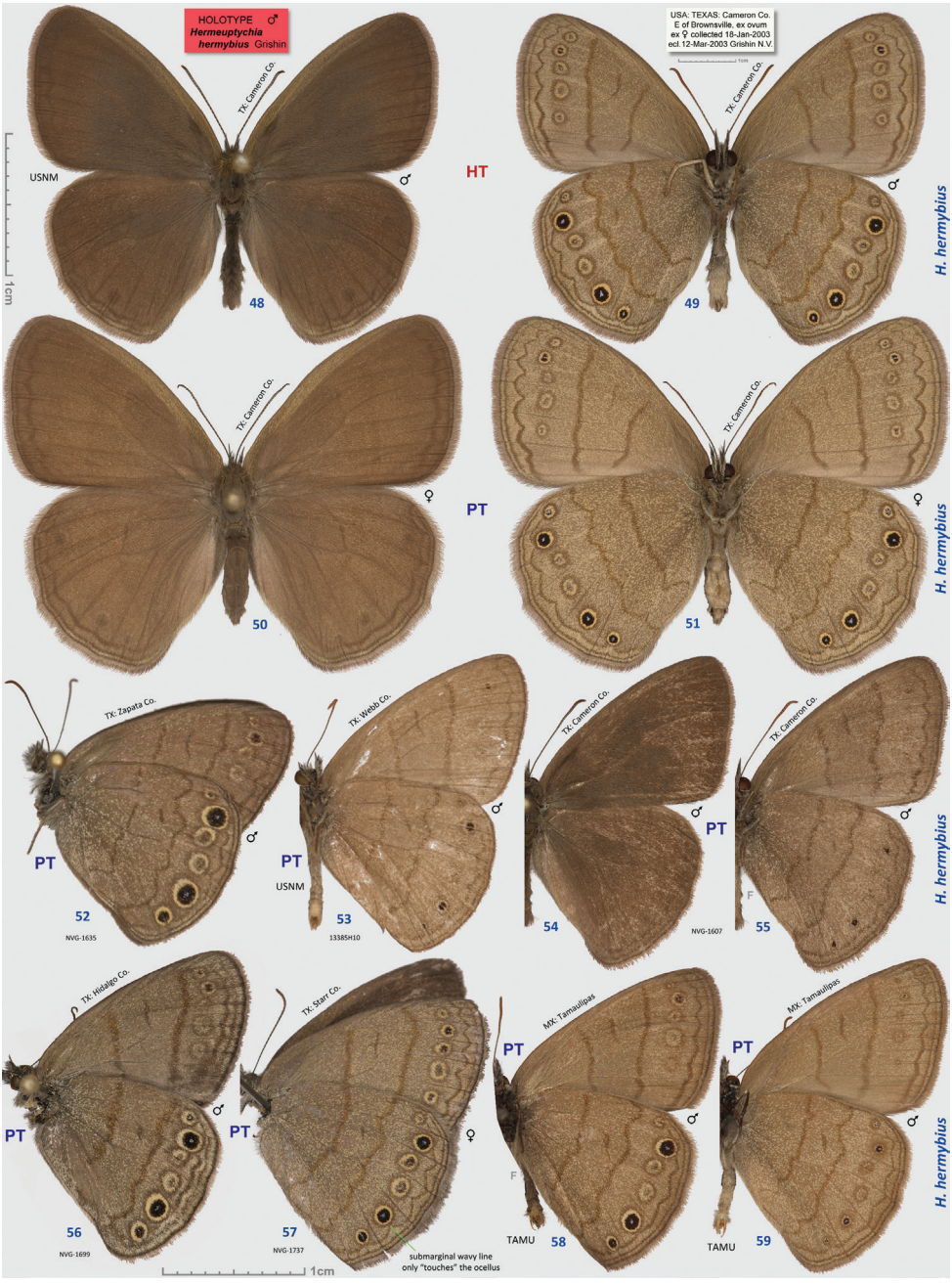
http://zoobank.org/B719B2F8-D0AD-4995-8372-6AA2FC2116E3
http://species-id.net/wiki/Hermeuptychia_hermybius
Figs 48–59, 60b, e, h, k, 61b, 62a–m, 63 part, 64q–z, 66 part, 67 part, 70

Description. Male (n=56, Figs 48–49, 52–56, 58–59) – holotype forewing length = 16 mm. Forewing triangular, rounded at apex and tornus, costal and outer margins convex, inner margin almost straight, mildly concave mediad, two discal cell veins budged at bases, vein 2A thickened basad. Hindwing rounded, almost circular. Wings dorsally

dark-brown with sparse olive-beige overscaling and two darker-brown terminal lines. Wings ventrally pale-brown, paler towards inner margin of forewing, with extensive beige overscaling, particularly along veins in distal part in some specimens; submedial and postmedial darker- to rusty- and olive-brown lines and end-of-cell streak (smaller on hindwing) between them; hindwing postmedial line more undulate that in *H. sosybius*, with a stronger bend in M_1-M_2 cell; two terminal dark-brown evenly curved marginal lines, dark-brown sinuous submarginal line, more undulate than in *H. sosybius*, barely touching the eyespot in cell Cu_1-Cu_2, and row of submarginal eyespots basad of the sinuous line and posteriad of postmedial line, eyespots frequently reduced in size and are more uniformly sized than in *H. sosybius*; usually largest eyespots black-centered and pupiled with pale-blue scales: on forewing, eyespots about the same size, frequently larger posteriad, but eyespot in cell M_1-M_2 (usually not the largest in size) and eyespot in cell R_5-M_1 (in some specimens) black-centered (more eyespots black centered in some specimens); on hindwing, largest eyespots in cells M_1-M_2 and Cu_1-Cu_2, a smaller one in cell Cu_2-1A+2A, even smaller, but still black-centered and pale-blue pupilled in cell Rs-M_1, and two smallest, usually without black, but in some specimens pale-blue pupilled eyespots in cells M_2-M_3 and M_3-Cu_1. Fringes monochrome, a little paler than the ground color of wings. Head, palpi, thorax and abdomen dark-brown above, paler and mostly beige beneath. Antennae dark-brown above with pale scales at segments, orange-brown at the club, beneath beige basad, orange-brown in distal half. Legs brown with beige scales. Male genitalia (n=19, Figs 60b, e, h, k, 61b, 62a–m, 63 part) – typical for the genus, very similar to those of *H. sosybius*. Tegumen dome-like, rounded at margins. Uncus leaf-shaped in dorsal view, almost flat distally but convex basally in lateral view, with a well-developed thin, membranous carina in basal half; apex of uncus appears truncated in dorsal view and sides usually less concave than in *H. sosybius*. Gnathos arms thin, wide apart, divergent, about the same length as uncus. Valvae narrow, but typically broader than in *H. sosybius*, elongated with thin cuculli extending past gnathos usually farther than a quarter of their length; cucullus usually with four apical teeth; cucullus ventrally with inner medial bulge. Saccus about the same length as cucullus, narrow. Aedeagus elongated, bent around its middle, with a medium length phallobase. Female (n=45, Figs 50–51, 57) – similar to male in facies, with slightly more rounded wings and dorsally paler in color. Female genitalia (n=9, Fig. 64q–z) as in *H. sosybius*, with pale, yellowish, weakly sclerotized and broad, rounder anteriad, cup-like antrum slightly asymmetric to the left. Ostium bursae ellipsoidal, its ventral margin shorter or equal to dorsal margin. Ductus and corpus bursae each in length similar to antrum; corpus bursae with two signa, spines in a signum narrow, leaf-shaped, placed in three to five irregular rows.

Barcode sequences. Full length DNA barcodes were obtained for 19 paratypes (GenBank accessions: KJ025569–KJ025587). The most common haplotype present in 17 sequences (including all 5 barcoded siblings of the holotype) is exemplified by the voucher NVG-1603, Genbank accession KJ025569, 658 base pairs:

AACTTTATATTTTATTTTTGGTATTTGAGCAGGAATAATTGGAACAT-
CATTAAGTTTAATTATTCGAATAGAGTTAGGTAATCCAGGATTTT-



Figures 48–59. *Hermeuptychia hermybius.* **48–49** holotype, others are paratypes, data in text and Table 1. Sexes and DNA or genitalia voucher codes, or data: **50–51** ♀ USA: Texas: Cameron Co., Brownsville, ex ovum, eclosed 2-Apr-2003, leg. N. V. Grishin **52** ♂ NVG-1635 **53** ♂ 13385H10 **54–55** ♂ NVG-1607 **56** ♂ NVG-1699 **57** ♀ NVG-1737 **58** ♂ NVG130104-23 **59** ♂ NVG130104-24. Dorsal wing surfaces are in **48, 50, 54** others are ventral. Labels are shown for the holotype and are reduced 2.5-fold compared to specimens as indicated by a smaller scale bar. "F" specifies mirror image (left-right inverted).

TAATTGGAGATGACCAAATTTATAACACTATTGTTACAGCCCATGCTTT-
TATTATAATTTTTTTTTATAGTAATACCTATTATAATTGGAGGATTTGG-
TAATTGACTTATTCCTTTAATATTAGGAGCTCCTGATATAGCTTTCC-
CACGTATAAATAATATAAGATTTTGATTATTACCCCCATCTTTAATTTT-
ATTAATTTCTAGTAGTATTGTAGAAAATGGAAGTGGAACAGGATGAACT-
GTTTACCCCCCTCTTTCATCTAATATTGCCCATAGAGGTTCTTCAGTA-
GATTTAGCAATTTTTTCTCTTCATTTAGCTGGAATTTCATCAATTTTAG-
GAGCCATTAATTTTATTACAACAATTATTAATATACGAATTAATAATA-
TATCTTATGATCAAATACCTTTATTTATTTGAGCTGTAGGAATTACA-
GCTCTTCTTTTACTTCTCTCATTACCTGTTTTAGCAGGAGCTATTAC-
CATACTTCTTACTGATCGAAATTTAAATACATCATTTTTTGACCCT-
GCAGGAGGAGGAGATCCTATTTTATATCAACATTTATTT

The 2 remaining sequences were identical to each other (Fig. 66b) and differed from the sequence shown above by a single base pair (0.15%). Barcode from the oldest and westernmost specimen (TX: Laredo, 15-Apr-1949) was additionally verified with both DNA ID tags as described in Materials and methods section and confirmed to be this species.

Type material. Holotype: ♂, has the following two rectangular labels: white printed - || USA: TEXAS: Cameron Co. | E of Brownsville, ex ovum | ex ♀ collected 18-Jan-2003 | ecl. 12-Mar-2003 Grishin N.V. ||; red printed - || HOLOTYPE ♂ | *Hermeuptychia* | *hermybius* Grishin ||. The holotype is illustrated in Figs 48–49. Upon publication, the holotype will be deposited in the National Museum of Natural History, Smithsonian Institution, Washington, DC (USNM). **Paratypes:** 55 ♂♂ and 45 ♀♀, from USA: Texas, unless indicated otherwise. Of these, 9 ♂♂ and 12 ♀♀ are siblings of the holotype read from ova, with the same data, their sexes, eclosion dates and GenBank accessions|DNA voucher numbers|genitalia codes (where available, and in this format for other paratypes) are: 1 ♀ 8-Mar-2003; 1 ♂ 9-Mar-2003, KJ025572|NVG-1610|NVG131017-02 (Fig. 62b); 2 ♂♂ and 1 ♀ 9-Mar-2003; 1 ♂ and 1 ♀ 10-Mar-2003; 1 ♂ and 1 ♀ 11-Mar-2003; 3 ♂♂ 12-Mar-2003; 1 ♀ 14-Mar-2003, KJ025573|NVG-1611|NVG131017-03 (Fig. 64s–t); 1 ♀ 15-Mar-2003; 1 ♀ 16-Mar-2003, KJ025574|NVG-1612|NVG131017-04 (Fig. 64u–v); 1 ♀ 17-Mar-2003, KJ025569|NVG-1603|NVG130927-17 (Fig. 64q–r); 2 ♀♀ 17-Mar-2003; 1 ♀ 21-Mar-2003; 1 ♂ 30-Mar-2003, KJ025571|NVG-1609|NVG131017-01 (Fig. 62a); 1 ♀ 2-Apr-2003 (Figs 50–51). Other paratypes are: 1 ♂ ibid., collected on wing 18-Jan-2003, KJ025570|NVG-1607|NVG130927-18 (Figs 54–55, 60b, e, h, k). 1 ♀ Cameron Co., E of Brownsville, 19-Oct-1997, leg. N. V. Grishin, KJ025575|NVG-1628|NVG131017-05. 1 ♂ Cameron Co., Brownsville, {10-13}-Mar-1979, leg. T. Friedlander, NVG140104-01 [TAMU] (Fig. 62c). 1 ♂ (06-Jun-2007) 1 ♀ (07-Jun-2007) Cameron Co., Los Fresnos, Ted Hunt & Loop Rd., leg. William R. Dempwolf. 4 ♀♀ Hidalgo Co., 1.5 air mi SE of Relampago, Rio Rico Rd., 26.07 -97.891, 21 m, 13-Jun-2013, leg. W. R. Dempwolf; 2 ♂♂ ibid., 19-Oct-2013, KJ025577|NVG-1698|NVG131229-04 (Fig. 62d) and KJ025578|NVG-1699|NVG131229-05 (Figs 56, 62e); 1 ♀ ibid., 19-Oct-2013, KJ025576|NVG-1695|NVG131229-03 (Fig. 64w–x); 3 ♂♂ 4 ♀♀ ibid., 19-Oct-2013; 2 ♂♂ 4 ♀♀

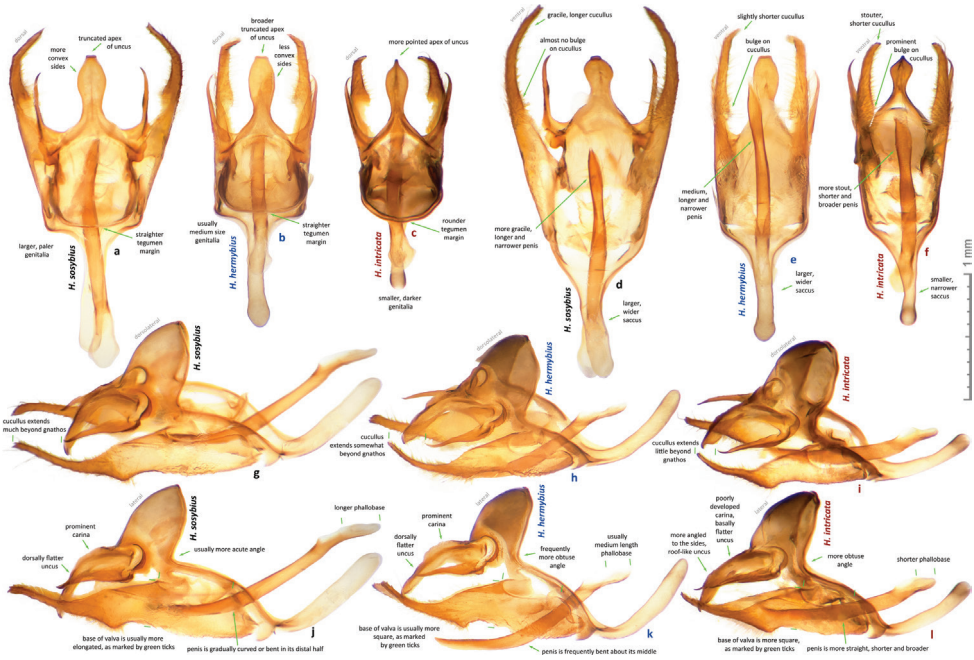


Figure 60. Male genitalia of *Hermeuptychia* from USA: Texas. **a, d, g, j** *H. sosybius*, Fort Bend Co., Brazos Bend State Park, Horseshoe Lake trail, 29°22'54.96" –95°36'41.06", 15 m, 17-Aug-2013, leg. N. V. Grishin, DNA voucher NVG-1542, genitalia NVG130927-03 (forewing length 15 mm) **b, e, h, k** *H. hermybius* sp. n. paratype, Cameron Co., E of Brownsville, 18-Jan-2003, leg. N. V. Grishin, DNA voucher NVG-1607, genitalia NVG130927-18 (specimen Figs 54–55, forewing length 15.5 mm) **c, f, i, l** *H. intricata* sp. n. holotype, Fort Bend Co., Brazos Bend State Park, near Hale Lake, 29°22'48.27" –95°35'05.02", 16 m, 17-Aug-2013, leg. N. V. Grishin, DNA voucher NVG-1560, genitalia NVG130927-14 [USNM] (specimen Figs 22–23, forewing length 16.5 mm). Views: **a**–**b** dorsal, perpendicular to the tegumen-uncus-gnathos plane **c**–**d** ventral, perpendicular to the plane of saccus and valvae (appears larger than dorsal view due to different projection axis) **e**–**f** right dorsolateral **g**–**h** right lateral. All images are to scale. Diagnostic characters are indicated on images. Note that *H. intricata* with larger than *H. sosybius* wings has smaller genitalia.

ibid., 21-Oct-2013; 3 ♂♂ ibid., 24-Oct-2013. 1 ♀ TX: Starr Co., Rio Grande City, Fort Ringgold, 26.3707 -98.8064, 45 m, 12-Nov-2010, leg. W. R. Dempwolf; 1 ♀ ibid., 13-Jun-2013; 1 ♂ ibid., 20-Oct-2013, KJ025580|NVG-1714|NVG131229-07 (Fig. 62f); 1 ♀ ibid., 20-Oct-2013, KJ025579|NVG-1712|NVG131229-06; 2 ♂♂ ibid., 20-Oct-2013; 1 ♂ ibid., 23-Oct-2013; 2 ♂♂ 1 ♀ ibid., 9-Nov-2013. 2 ♂♂ Starr Co., Roma, S of Roma International Bridge, 26.4035 -99.0175, 50 m, 20-Oct-2013, leg. W. R. Dempwolf, KJ025581|NVG-1726|NVG131229-08 (Fig. 62g) and KJ025582|NVG-1727|NVG131229-09 (Fig. 62h); 8 ♂♂ 7 ♀♀ ibid., 20-Oct-2013. 1 ♀ Starr Co., Roma Creek, Hwy 650/Hwy 83, 29-Oct-2007, leg. W. R. Dempwolf. 2 ♀♀ Starr Co., 0.5 mi S of Fronton, 26.399 -99.085, 50 m, 20-Oct-2013, leg. W. R. Dempwolf, KJ025583|NVG-1735|NVG131229-10 and KJ025584|NVG-

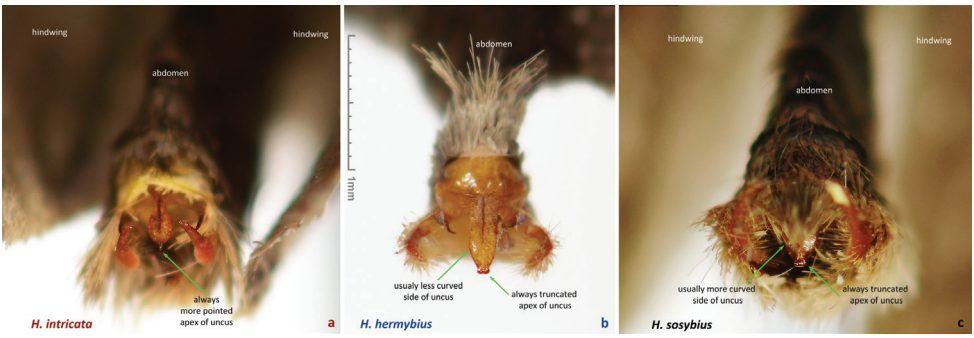


Figure 61. Dorsoposterior view of male abdomens of *Hermeuptychia* from USA: Texas. **a** *H. intricata*, DNA voucher NVG-1548 (mirror image, i.e. left-right inverted) **b** *H. hermybius*, DNA voucher NVG-1635 (also shown in Fig. 62j, specimen Fig. 52) **c** *H. sosybius*, DNA voucher NVG-1553. Data in Table 1. Scales are brushed off the abdomen tip to expose distal parts of genitalia. The easiest to observe character (the shape of the distal end of uncus) is indicated.

1737|NVG131229-11 (Figs 57, 64y–z); 7 ♂♂ 3 ♀♀ ibid., 20-Oct-2013. 1 ♂ Starr Co., Salineno @ Rio Grande, 26.51463 -99.11633, 53 m, 23-Oct-2013, leg. W. R. Dempwolf, KJ025585|NVG-1747|NVG131229-12 (Fig. 62i). 1 ♂ Zapata Co., San Ygnacio @ Rio Grande, 92 m, 7-Oct-2007, leg. N. V. Grishin, KJ025586|NVG-1635|NVG131017-12 (Figs 52, 61b, 62j). 1 ♂ Webb Co., Laredo, 15-Apr-1949, leg. E. L. Todd KJ025587|13385H10|NVG131102-53 [USNM] (Figs 53, 62k). 1 ♂ Mexico: Tamaulipas: Rt. 101 at Rio Corona, 1-Jan-1980, leg. P. W. Kovarik & D. S. Bogar, NVG140104-04 [TAMU]. 1 ♂ Mexico: Tamaulipas: El Canindo, nr. Ejido San José, 7.5 km W Gómez Farías, 1400 m, {19-21}-Jul-1994, leg. C. Cate & T. Riley, NVG140104-67 [TAMU]. 2 ♂♂ Mexico: Tamaulipas: Ciudad Mante, Los Arcos Ct., 19-Dec-1973, leg. R. O. & C. A. Kendall, NVG140104-22 and NVG130104-23 [TAMU] (Figs 58, 62m); 1 ♂ ibid., 28-Jan-1995, ex larva, foodplant *Panicum maximus* Jacq., NVG140104-24 [TAMU]. 1 ♂ Mexico: Tamaulipas: Quintero cave [22.6333 -99.0333], 7-Jan-1974, leg. R. O. & C. A. Kendall, NVG130104-24 [TAMU] (Figs 59, 62l). 1 ♂ 1 ♀ Mexico: San Luis Potosí: El Salto Falls, 30-Dec-1979, leg. P. W. Kovarik & D. S. Bogar, NVG140104-03 and NVG140104-02 [TAMU].

Type locality. USA: Texas: Cameron County, east of Brownsville. It is a shaded area covered in Guinea grass (*Panicum maximus*), situated near a ravine and overgrown with taller trees.

Etymology. The name is a fusion of two words: herm[es] beginning and [sos] ybius ending. It symbolizes that this species traditionally and previously regarded as *H. hermes* is phylogenetically closer to *H. sosybius*, and yet is distinct from it. The resulting word is unique and currently unknown to internet search engines, which is expected to ease its searches. The name is a noun in apposition.

Distribution. This species is currently recorded from the lower Rio Grande Valley region of Texas along the Rio Grande from Laredo to the Gulf coast (Webb, Za-

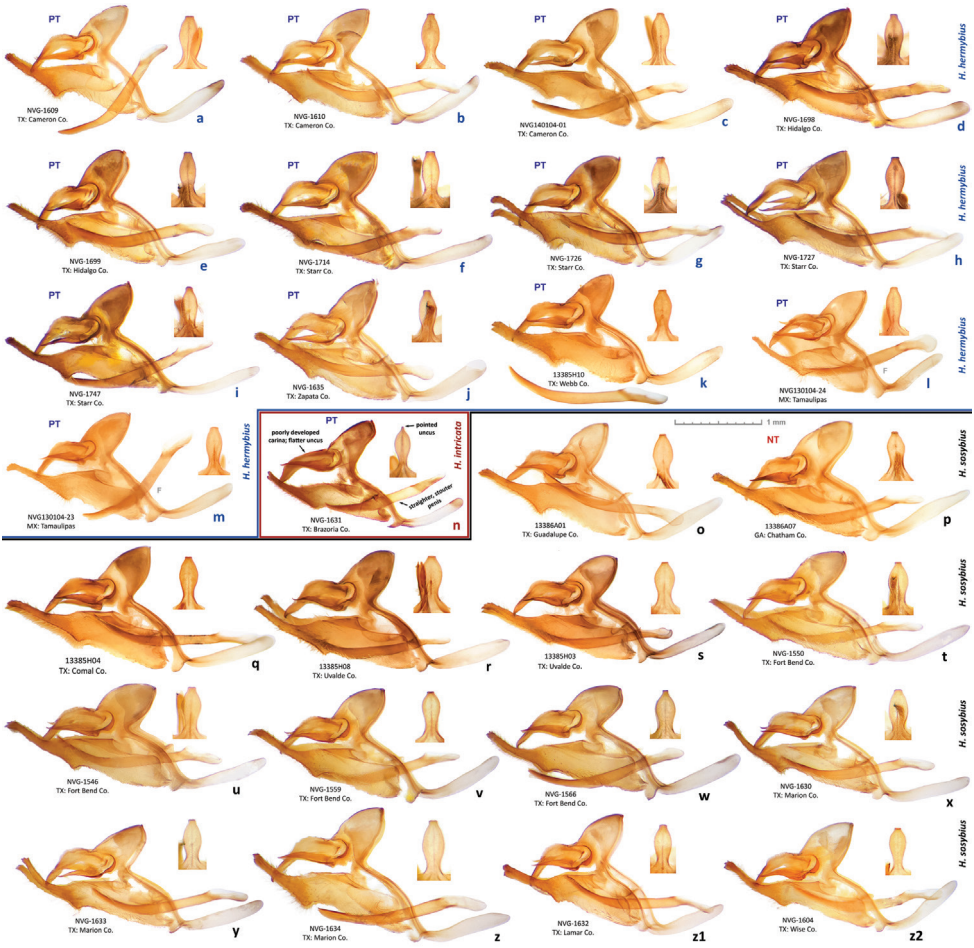


Figure 62. Variation in male genitalia of *H. hermybius* and *H. sosybius*. **a**–**m** *H. hermybius* paratypes, DNA (or genitalia, where DNA sequence is not available, and full data for these given) voucher codes: **a**. NVG-1609 **b** NVG-1610 **c** Texas: Cameron Co., Brownsville {10-13}-Mar-1979, T. Friedlander, NVG140104-01 **d** NVG-1698 **e** NVG-1699 (specimen Fig. 56) **f** NVG-1714 **g** NVG-1726 **h** NVG-1727 **i** NVG-1747 **j** NVG-1635 (also shown in Fig. 61b, specimen Fig. 52) **k** 13385H10 (specimen Fig. 53) **l**–**m** Mexico: Tamaulipas, leg. R. O. & C. A. Kendall: **l** Quintero cave, 7-Jan-1974, NVG130104-24 (specimen Fig. 59) **m** Ciudad Mante, Los Arcos Ct., 19-Dec-1973, NVG130104-23 (specimen Fig. 58) **n** *H. intricata* paratype, NVG-1631 (specimen Figs 28–29), diagnostic characters are indicated on the image **o**–**z2** *H. sosybius*: **o** 13386A01 (specimen Fig. 47) **p** 13386A07, neotype (specimen Figs 9–11) **q** 13385H04 **r** 13385H08 (specimen Fig. 46) **s** 13385H03 **t** NVG-1550 **u** NVG-1546 **v** NVG-1559 **w** NVG-1566 **x** NVG-1630 **y** NVG-1633 **z** Texas: Marion Co., W of Caddo Lake, 5-Apr-1997, leg. N. V. Grishin, NVG-1634 **z1** NVG-1632 **z2** Texas: Wise Co., LBJ National Grassland, ex ovum, eclosed 3-Aug-1998, leg. N. V. Grishin, NVG-1604. **c**, **l**, **m** are in TAMU and **o**–**s** are in USNM collections. Data for most specimens are in Table 1, text, or specified above. Complete genitalia are shown in lateral view, and dorsal view of uncus is shown above and to the right of each specimen. Aedeagus is shown below in **k** DNA (or genitalia, where DNA sequence is not available) voucher codes and general localities are indicated below each genitalia image. "F" specifies mirror image (left-right inverted).

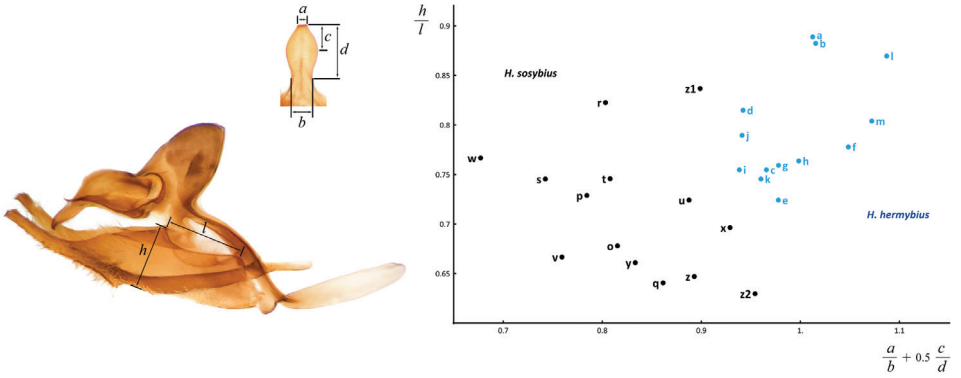


Figure 63. Morphometric differences between male genitalia of *H. sosybius* (black) and *H. hermybius* (blue). Measurements used are marked on dorsal view of uncus (top left) and on lateral view of complete genitalia (bottom left): **a** width of uncus at the apex **b** width of uncus at the narrowest point near the base ("neck" at the joint with tegumen) **c** distance from the uncus apex to the cross-section at the widest point **d** distance from the uncus apex to the cross-section at the narrowest point near the base **l** length of valval dorsal "window" **h** height of valva (in lateral view) at the end of the dorsal "window", direction of height measurement is perpendicular to the direction of length measurement. Measurements of genitalia shown in Fig. 62 are plotted on the right. Horizontal axis combines all uncus measurements into a formula *a*/*b*+0.5**c*/*d* and vertical axis shows measurements of valva as *h*/*l*. Each point corresponds to a specimen and a letter next to it is the same one that denote its genitalia in Fig. 62.

pata, Starr, Hidalgo, and Cameron Counties, Fig. 67) and in neighboring Mexico (Tamaulipas, San Luis Potosí).

Diagnosis. In wing pattern, the new species is most similar to *H. sosybius*, but typically can be differentiated from it by: (a) eyespots that are not only smaller, but also more uniform in size, i.e. out of 5 forewing eyespots, 4 (except the one near costa) are usually about the same size, and the eyespot that is black-ringed in most specimens (second from costa) is typically not the largest (this eyespot is frequently the largest in *H. sosybius*), but the next-to-last eyespot (4th from the costa) is usually the largest one; (b) more undulate postmedial line on ventral hindwing, that frequently strongly bulges basad by the largest eyespot near apex (in cell M_1-M_2); (c) more undulate submarginal sinuous line, which on ventral hindwing barely touches the largest eyespot near the tornus (in cell Cu_1-Cu_2, second eyespot from tornus, indicated in Fig. 57)–this line is usually fully merged with this eyespot border for some distance in *H. sosybius*. Wing-based identification is not absolute due to extensive pattern variation in both species.

In male genitalia, the new species is also closest to *H. sosybius* and should be attributed to the same morphogroup 4 of Seraphim et al. (2014). It differs from *H. sosybius* in the following trends (Figs 60–61): (1) uncus is less convex and narrower on the sides in dorsal (or ventral) view, with a broader truncated apex, the width at the apex is usually more than 2/3 of the width at the narrowest point near the base (Figs 60b, e, 61b); (2) valva is typically "higher" in lateral view (dorso-ventral direction), more square at the base (Fig. 60k) and is less extended (Fig. 60h); (3) aedeagus is somewhat broader

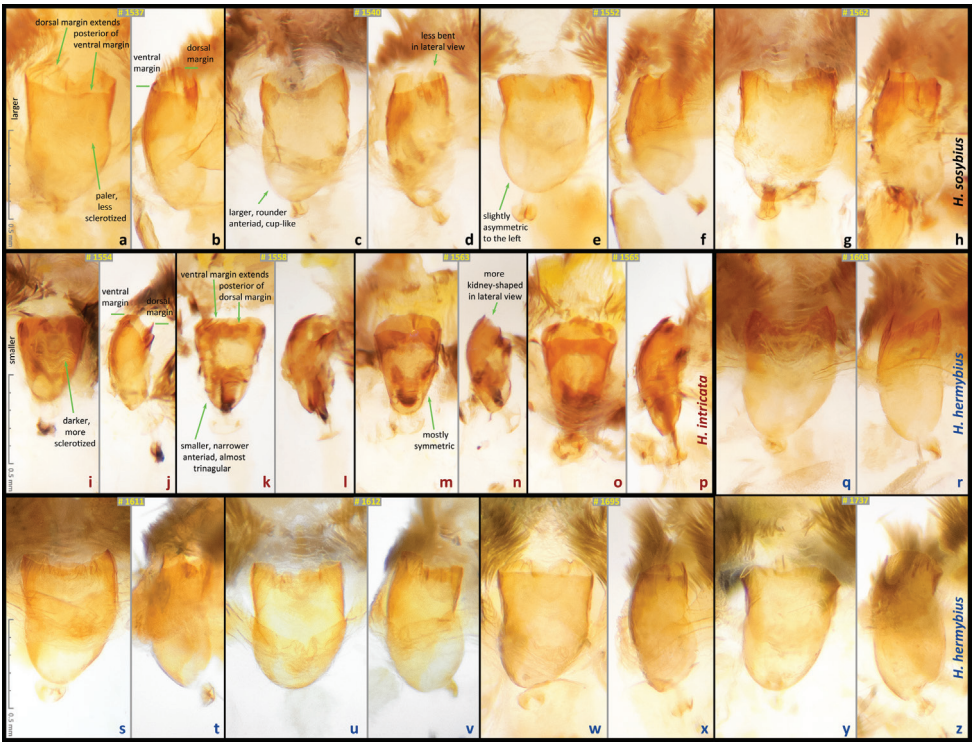


Figure 64. Antrum in female genitalia of *Hermeuptychia* from USA: Texas. **a**–**h** *H. sosybius*, Fort Bend Co., Brazos Bend State Park, 17-Aug-2013, leg. N. V. Grishin: **a**–**f** is from Horseshoe Lake trail, 29°22'54.96" –95°36'41.06", 15 m and **g**–**h**. is from near Hale Lake, 29°22'48.27" –95°35'05.02", 16 m; DNA voucher|genitalia dissection codes are: **a**–**b** NVG-1537|NVG130927-01 **c**–**d** NVG-1540|NVG130927-02 (specimen Fig. 12) **e**–**f** NVG-1552|NVG130927-06 **g**–**h** NVG-1562|NVG130927-10 **i**–**p** *H. intricata* sp. n. paratypes, Fort Bend Co., Brazos Bend State Park, near Hale Lake, 29°22'48.27" –95°35'05.02", 16 m, 17-Aug-2013, leg. N. V. Grishin; DNA voucher|genitalia dissection codes: **i**–**j** NVG-1554|NVG130927-07 (specimen Fig. 24) **k**–**l** NVG-1558|NVG130927-08 **m**–**n** NVG-1563|NVG130927-11 **o**–**p** NVG-1565|NVG130927-12 (specimen Fig. 25) **q**–**z** *H. hermybius* sp. n. paratypes **q**–**r** Cameron Co., E of Brownsville, ex ovum ex ♀ captured on 18-Jan-2003, eclosed on 17-Mar-2003, leg. N. V. Grishin, NVG-1603|NVG130927-17 **s**–**t** ibid., eclosed on 14-Mar-2003, NVG-1611|NVG131017-03 **u**–**v** ibid., eclosed on 16-Mar-2003, NVG-1612|NVG131017-04 **w**–**x** TX: Hidalgo Co., 1.5 air mi SE of Relampago, Rio Rico Rd., 26.07 -97.891, 21 m, 19-Oct-2013, leg. W. R. Dempwolf, NVG-1695|NVG131229-03 **y**–**z** Starr Co., 0.5 mi S of Fronton, 26.399 -99.085, 50 m 10-Oct-2013, leg. W. R. Dempwolf, NVG-1737|NVG131229-11 (specimen Fig. 57). Additional data for specimens and their DNA barcodes are in Table 1. In all images, posterior end is pointing up (i.e. ostium bursae is closer to the top of each image); **a, c, e, g, i, k, m, o, q, s, u, w, y** are in lateral view, others are in right ventrolateral view. All images are to scale. Diagnostic characters to tell between *H. sosybius* and *H. intricata* are indicated on images, each character was invariantly observed in all inspected samples of a species, but is indicated (for clarity) on a single image only. We failed to find characters distinguishing female genitalia of *H. hermybius* from *H. sosybius* and simply illustrate genitalic variation.

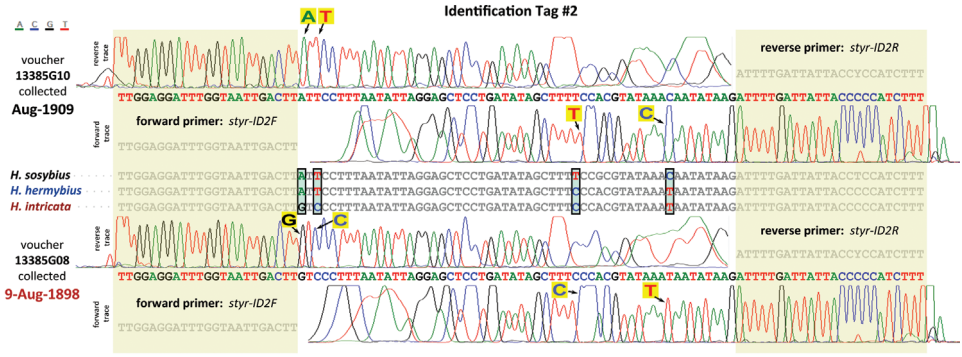
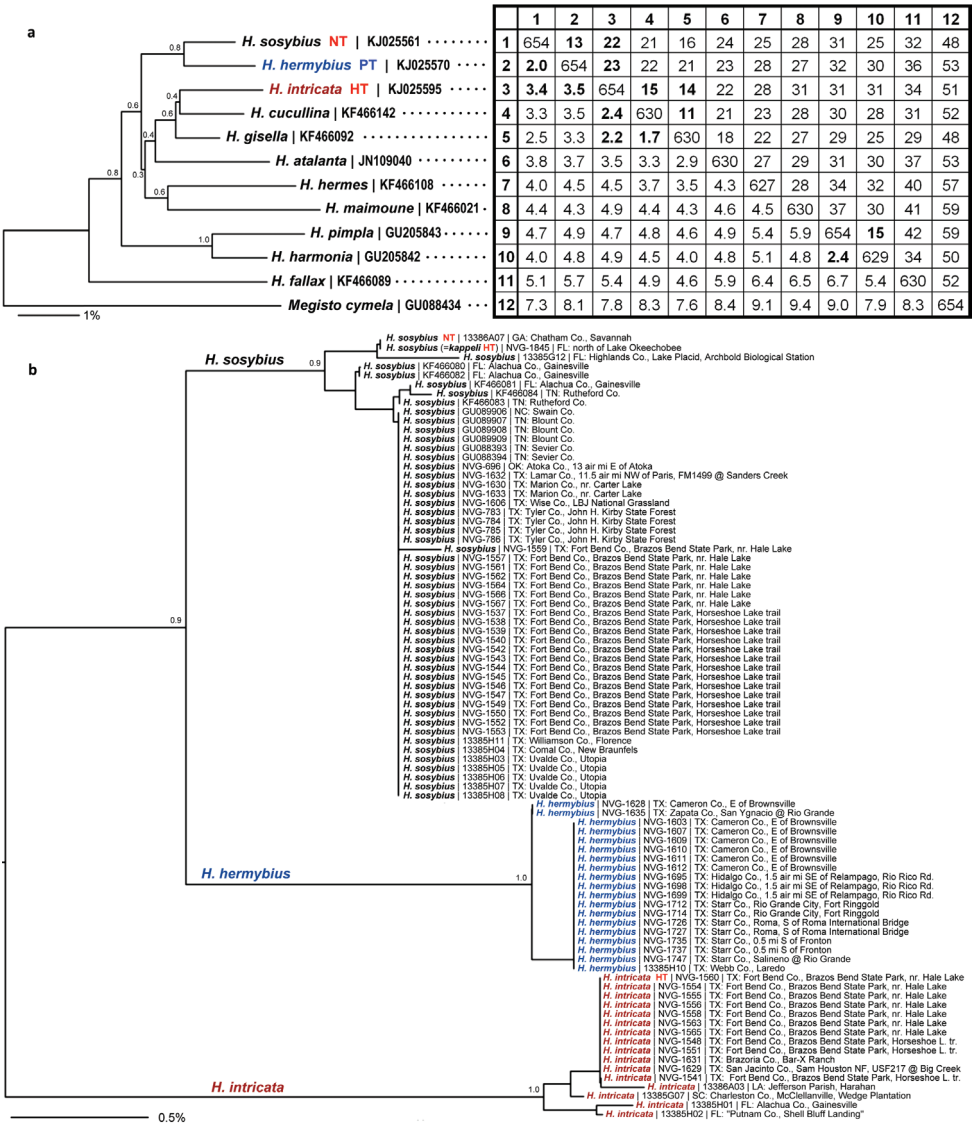


Figure 65. DNA ID tags of specimens that are over 100 years old. ID tag #2 is shown as an example. The tag region sequence alignment of the three species: *H. sosybius*, *H. hermybius*, and *H. intricata* is shown in the middle and positions at which sequences differ are highlighted in cyan and boxed. Each of the three species differs from the other two by at least 2 nucleotides, and *H. sosybius* is different from *H. intricata* by 4 nucleotides. Forward and reverse primer regions are shaded. DNA of the tag was amplified and sequenced in both forward and reverse directions from two over-100-years-old specimens from the same locality (SC: Clarendon Co.). Forward and reverse sequences traces for the first specimen are shown above the reference sequences and the two traces for the second specimen are shown below. It is clear from the traces that the specimen above (13385G10, Fig. 36) is *H. sosybius*, (A, T, T, & C at these 4 positions, no contamination seen) and the one below (13385G08, Fig. 33) is *H. intricata* (G, C, C, & T at these 4 positions and equally unambiguous traces). Nucleotides that identify each specimen are indicated in large letters on yellow background and arrows point to the trace peaks revealing these nucleotides. This strategy was applied to identify 12 very old specimens of three species in a random order and yielded unambiguous identifications for 11 of them. One sample appeared to be contaminated, and the traces showed the presence of several nucleotides in many positions. All 11 DNA-based identifications agreed with genitalic identifications.

and is frequently bent near its middle, with a medium length phallobase (Fig. 60e); (4) usually more obtuse angle formed by the tegumen and vinculum in lateral view (Fig. 60k). These characters are quite subtle, and as illustrated in Fig. 62 (compare panels a–m with panels o–z2) are subject to significant variation. In contrast, distinction of *H. intricata* (Fig. 62n for comparison) is always definitive and clear-cut. To evaluate the confidence of *H. hermybius* identification by male genitalia and to test the ability to differentiate this new species from *H. sosybius* by objective criteria, we resorted to morphometric analysis (Fig. 63). For simplicity, we have chosen to exploit only two trends listed above: (1) shape of uncus in dorsal view and (2) shape of valva base in lateral view. The shape of uncus was measured by the ratio of width at the apex (*a*) to the width at the narrowest point near the base (*b*), and by the ratio of the distance from apex to the widest point in cross-section (*c*) to the distance from apex to the narrowest point near the base (*d*). We noticed that both of these ratios tend to be smaller in *H. sosybius*. Instead of applying PCA or other similar data-driven technique, which may be biased by the data at hand (i.e. the resulting transformation would change with the dataset used), we combined these measurements in a data-independent transforma-



	1	2	3	4	5	6	7	8	9	10	11	12
1	654	**13**	**22**	21	16	24	25	28	31	25	32	48
2	**2.0**	654	**23**	22	21	23	28	27	32	30	36	53
3	**3.4**	**3.5**	654	**15**	**14**	22	28	31	31	31	34	51
4	3.3	3.5	**2.4**	630	**11**	21	23	28	30	28	31	52
5	2.5	3.3	**2.2**	**1.7**	630	18	22	27	29	25	29	48
6	3.8	3.7	3.5	3.3	2.9	630	27	29	31	30	37	53
7	4.0	4.5	4.5	3.7	3.5	4.3	627	28	34	32	40	57
8	4.4	4.3	4.9	4.4	4.3	4.6	4.5	630	37	30	41	59
9	4.7	4.9	4.7	4.8	4.6	4.9	5.4	5.9	654	**15**	42	59
10	4.0	4.8	4.9	4.5	4.0	4.8	5.1	4.8	**2.4**	629	34	50
11	5.1	5.7	5.4	4.9	4.6	5.9	6.4	6.5	6.7	5.4	630	52
12	7.3	8.1	7.8	8.3	7.6	8.4	9.1	9.4	9.0	7.9	8.3	654

Figure 66. DNA-derived data. **a** Analysis of named *Hermeuptychia* species **b** relationships between *Hermeuptychia* specimens from USA in a form of BioNJ (Dereeper et al. 2008) distance tree. **a** DNA barcode distance matrix is shown on the right and a BioNJ distance tree corresponding to it is on the left. The tree is rooted with *Megisto cymela* (Cramer, 1777) sequence. A more comprehensive tree that includes several specimens of each species (except those described herein) and their detailed analyses are given in Seraphim et al. (2014) and is not repeated here. Only a single representative sequence for each species is used in **a** for clarity. The scale bar corresponding to about 1% difference in sequences is placed below the tree. Bootstrap support values are shown by each node in the tree; values below 0.5 indicate possibly incorrect groupings. GenBank accessions (http://genbank.gov/) for sequences are given after species names. NT, PT and HT refer to neotype, paratype and holotype, respectively. Data for specimens are in Table 1. Specimens 4–11 were not examined and their identification follows that of the authors

tion. We used a weighted sum of the two ratios, with the weight of the second ratio arbitrarily set to half the weight of the first one: $a/b+0.5c/d$, since the ratio of widths (first ratio) seemed to tell the species apart better than the ratio of lengths (second ratio). The shape of the valva base in lateral view was quantified by the ratio of length of the dorsal "window" (less sclerotized, membranous and flat segment along dorsal side near the base) to the height of the valva at the distal end of the "window". These variables were measured and computed on a diverse sample of 27 genitalia illustrated in Fig. 62. The resulting plot (Fig. 63 on the right) separated the two species. Therefore these simple measurements could be used to tell between these two cryptic *Hermeuptychia* species by male genitalia. However, we were not able to find characters in female genitalia to differentiate the new species from *H. sosybius*.

Finally, the most confident identification is provided by DNA barcode sequences (Fig. 66) that show little variation within each species (most sequences are identical across the range, maximum difference below 1% in *H. sosybius*), but reveal a definitive 2% hiatus between central and south Texas populations (Figs 66–67). We selected all positions that were invariant in the barcode sample of each species but different between the two species as characters to differentiate *H. hermybius* from *H. sosybius*. The resulting 11 positions are listed in the format "k X (not Y)", where k is a sequential number of the position (numbering is from 1 to 658 for the barcode sequence shown above as a reference), X is a nucleotide in *H. hermybius* barcodes and Y is a nucleotide in *H. sosybius* barcodes: 64 T (not C), 73 G (not A), 82 T (not C), 118 C (not T), 133 C (not T), 235 C (not T), 238 A (not G), 364 C (not T), 436 C (not T), 526 A (not T), 616 C (not T). These positions distinguish the two species; however, some of the positions are expected to show variation when a larger sample of sequence is accumulated.

Life history. The holotype of the new species, along with 21 paratypes are specimen reared in the lab from ova obtained from a captive female. All life history stages are illustrated in Fig. 70, and could be compared to the images of *H. sosybius* life history (Fig. 69). Immature stages of both species are very similar and without larger sample it is difficult to derive solid conclusions about the differences. Nevertheless, the following observations were made. Natural foodplants seems to be *Panicum maximus* (Guinea grass) per R. O. Kendall & C. A. Kendall, who reared caterpillars found on

who performed sequencing studies and analyses (Peña et al. 2010, Silva-Brandão et al. 2011, Seraphim et al. 2014). Percent difference and the number of different nucleotides are shown below and above the diagonal in the matrix, respectively, and the length of each sequence segment (bp) used in the analysis is on the diagonal. Most instructive values discussed in the text are shown in bold font **b** GenBank accession numbers (those that start with letters G or K) for sequences retrieved from GenBank or DNA voucher numbers (those that start from a number or letter N) for sequences obtained in this study and locality data for specimens are given for each sequence. Further details about the specimens are provided in Table 1. *H. sosybius* specimens with sequences obtained from GenBank were not examined and their identification follows that of the authors who performed sequencing studies (Murray and Prowell 2004, Hebert et al. 2010 & Seraphim et al. 2014). All *H. hermybius* and *H. intricata* specimens are paratypes, except the holotype marked with "HT". Scale bar shown below indicates about 0.5% difference.

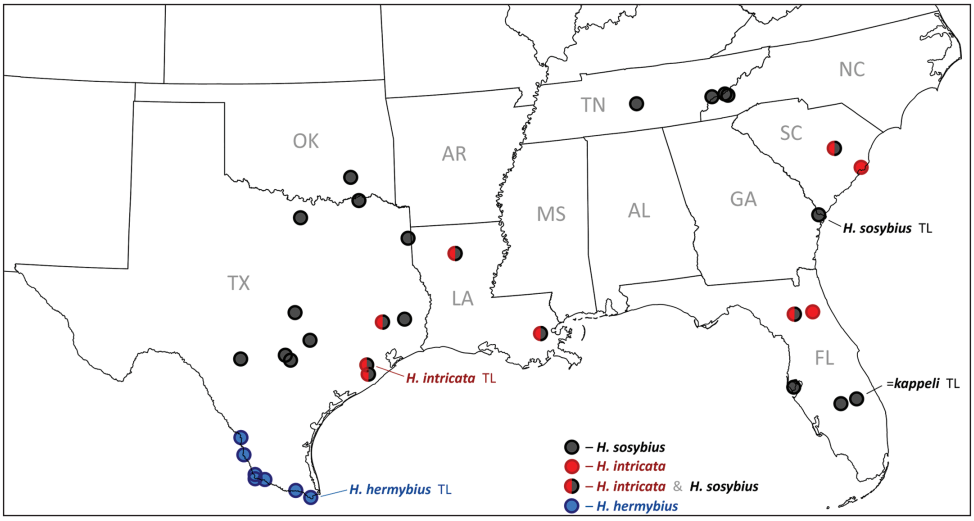


Figure 67. USA localities of *Hermeuptychia* specimens with available DNA barcode information. Color of circles corresponds to species: *H. sosybius*–black; *H. hermybius*–blue, *H. intricata*–red, split red/black circles mark localities where both *H. sosybius* and *H. intricata* were recorded. Type localities are indicated with a corresponding name followed by "TL". *Hermeuptychia hermes kappeli* was treated as a junior subjective synonym of *H. sosybius* by Pelham (2008). DNA barcode of *H. h. kappeli* holotype is 100% identical with the barcode of *H. sosybius* neotype. DNA barcode amplification failed for *H. intricata* specimen from LA: Jonesboro and for *H. sosybius* specimen from TX: Brazoria Co., and their identification is based on genitalia only. Specimens from all localities except those from TN and NC (data from GenBank, specimens not inspected) and from FL: St. Petersburg (specimen lacked abdomen) were dissected, and genitalia-based identification agreed with DNA barcode-based identification in all cases (see Fig. 66).

this grass in Mexico: Tamaulipas [TAMU collection]. This plant is also common in the lower Rio Grande Valley and is ubiquitously present where *Hermeuptychia* adults were encountered. Caterpillars hatched from eggs in captivity readily accepted *Cynodon dactylon* (L.) Pers. (Bermuda grass) and were successfully reared on it. Both *H. sosybius* and *H. hermybius* caterpillars go through four instars prior to pupation, and the first instar has black head capsule (Figs 69b–d, 70b–c). In subsequent instars, head capsule is green and round, without horns and projections (Figs 69e–m, 70d–m). Caterpillars of both species typically rest below leaves on loosely made silk pads, frequently in pairs, when two caterpillars face each other "head-to-head" (Figs 69h, 70c). When disturbed, caterpillars first curl into a C head-to-tail while legs being attached to the leaf (Figs 69f, j, 70e), then to a full O, head-to-legs (Fig. 70g). White dorsolateral spots in ultimate instar seem to be more pronounced in *H. hermybius* than in *H. sosybius* (compare Fig. 70g–k with 69k). Pupae of *H. hermybius* were stronger patterned with brown on the sides (Fig. 70o) than those of *H. sosybius* from two distant-from-each-other Texas localities (Fig. 69n–o), and some *H. hermybius* pupae were brown in color (Fig. 70n).

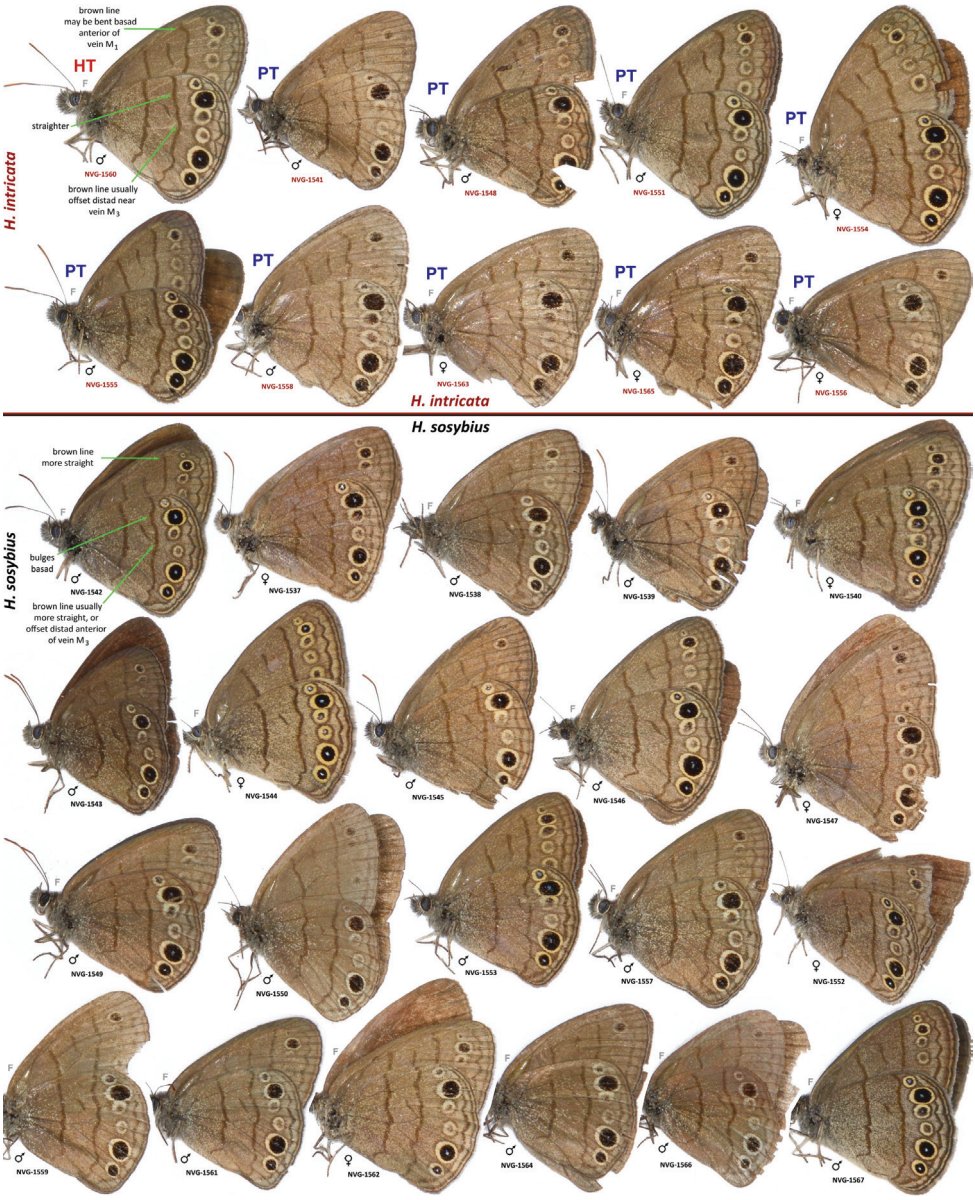


Figure 68. DNA-barcoded *Hermeuptychia* specimens from USA: Texas: Fort Bend Co., Brazos Bend State Park. *H. intricata* is above the line and *H. sosybius* is below the line, photographed prior to removal of body parts for DNA extraction. DNA voucher codes (see Table 1 for data) are shown below each specimen. Hypothetical field marks are indicated on the first specimen of each species. NVG-1537–NVG-1553 are from Horseshoe Lake trail, 29°22'54.96" –95°36'41.06", 15 m; and NVG-1554–NVG-1567 are from near Hale Lake, 29°22'48.27" –95°35'05.02", 16 m, all collected on 17-Aug-2013. Both species are present in each locality. Images are scaled approximately. "F" specifies mirror image (left-right inverted).

Figure 69. Life history of *H. sosybius*. USA: TX: Brazoria County, Bar-X Ranch, Rd. 971N, 29.13252 -95.58340, ex ovum ex ♀ collected on 4-Mar-2000, except **o**, which is TX: Wise Co., LBJ National Grassland. **a** ovum, 6-Mar-2000 **b–d** 1st instars, photographed on 14- 14- & 16-Mar-2000, respectively **e–g** 2nd instars photographed on 21- 19- & 21-Mar-2000 **e**, **f** are just after molt, shed larval skins are behind and 1st instar head capsule (black) is on the left in **e**, **f** is in a curled position adopted when disturbed **h** pre-molt quiescent 2nd instar larvae in a typical "head-to-head" resting position, 24-Mar-2000 **i–j** 3rd instars, 24- & 27-Mar-2000 **k–l** 4th (ultimate) instars, ♂♂, 3- & 6-Apr-2000 **l** close to pupation, note the color and shape change **m** prepupa, 6-Apr-2000 **n–p** pupae, 9-Apr-2000, 8-Aug-1998, & 17-Apr-2000 **o** is from Wise Co., wing color is starting to develop **p** near eclosion, dark adult is seen through semi-transparent pupal cuticle. Most images show different individuals. Images **a–g** are enlarged 2 times (scale on **f**) compared to the rest (scale on **l**).

Figure 70. Life history of *H. hermybius*. USA: TX: Cameron County, E of Brownsville, ex ovum ex ♀ collected on 18-Jan-2003. **a** ovum, 23-Jan-2003 **b**–**c** 1st instars, photographed on 30-Jan & 1-Feb-2003, respectively **b** prior to feeding, thus is white in color, **c** shows two caterpillars in a typical "head-to-head" resting position **d** 2nd instar, 10-Feb-2003 **e**–**f** 3rd instars 14- & 15-Feb-2003 **g**–**k** 4th (ultimate) instars, 25- 28- 26- 26- & 25-Feb-2003 **g** is in a curled position adopted when disturbed; **l**–**m** prepupae, 26- & 23-Feb-2003 **n**–**p** pupae, 10-Mar 25-Feb & 4-Mar-2003 **n** is a brown form, shed larval skin is still attached near cremaster in **o** and **p** and is hanging behind the pupa in **n**, **p** near eclosion, dark adult is seen through semi-transparent pupal cuticle. Most images show different individuals, those that eclosed are paratypes. Images **a**–**d** are enlarged 2 times (scale on **d**) compared to the rest (scale on **k**).

Discussion

We pose and answer some questions that are likely to arise regarding our description of *H. intricata* and *H. hermybius*.

Can *H. intricata* be identified by wing patterns alone? The sample of 20 barcoded and dissected *H. intricata* specimens from the type series (plus 2 more dissected paratypes without barcode sequences) is too small to judge with confidence. At the moment, it seems risky to accept wings-only identification. However, comparative analysis of wing patterns suggests the following three characters that should be investigated further (marked on representative specimens in Fig. 68). First, the postmedial dark-brown line on the ventral hindwing in *H. intricata* bulges distad near the vein M_3, in between the two smaller eyespots, closer to the posterior eyespot (e.g., compare Figs 32–47, 68). The line is more straight, or sinuous in *H. sosybius*, but frequently with a bulge anterior of the vein M_3 (closer to large eyespot that is nearer the apex). Second, this line is relatively straight anterior of vein M_2 in *H. intricata*, but is frequently bulged basad in *H. sosybius*. Third, in some *H. intricata* specimens, the postmedial dark-brown line on ventral forewing bends basad toward the costa (Fig. 68). More precisely, this line bends slightly distad towards the largest eyespot (in cell M_1-M_2) and then bends basad from near vein M_1 (just anteriad of the largest eyespot) to costal margin. In *H. sosybius*, this line is typically straight or even bends distad towards costa. If the line bends basad, the bend is more gradual and begins posterior of M_1 vein (posteriad of the largest eyespot). However, in some *H. intricata* specimens this bend is not present (Figs 29, 31, 34, 41). If these hypothesized field marks are indeed meaningful, then an individual photographed by Tveten and Tveten (1996: 186) – photograph reproduced with modifications in Brock and Kaufman (2003: 231, rightmost illustration) – is *H. intricata*. Judging from the title of the Tvetens' book, it was photographed in east Texas near Houston, which matches the expected distribution range of this species. Interestingly, another individual shown in Tveten and Tveten (1996: 178) on a full page photograph appears to be typical *H. sosybius*. To facilitate further identification of field marks, we illustrate almost the entire type series of *H. intricata* (Figs 22–35, 40–43, 68), and all DNA barcoded specimens of *H. intricata* and *H. sosybius* from the Brazos Bend State Park, Texas (Fig. 68).

Are DNA barcode sequences necessary for confident identification of *H. intricata*? Although the DNA barcoding study has been instrumental in this project, we believe that its conclusions would hold without the knowledge of barcode sequences. Although we first noticed the difference in DNA barcodes, had we dissected the specimens prior to that, the presence of the two species (*H. sosybius* and *H. intricata*) and distinction between them would have become equally clear. We think male and female genitalia offer solid diagnostic characters that are sufficient for confident identification. These characters are numerous, are listed in the diagnosis above, and are illustrated in Figs 60–62 & 64. Therefore, DNA barcode sequences are not required for confident identification of this new species. Nevertheless, barcodes were very valuable to suggest that south Texas *H. sosybius*-like populations with typically smaller, more uniform-

ly-sized eyespots and more undulate ventral hindwing lines are not conspecific with eastern USA populations, but represent a new species, *H. hermybius*. DNA barcodes were equally valuable to confirm that the name *H. hermes* should be best reserved for a South American species (Seraphim et al. 2014).

Should *H. intricata* be described now from a small sample? We believe that a description of a new species is an invitation to study it further. The discovery of a new butterfly species in the USA, especially rather distant evolutionarily from other members of the fauna (closest relatives in Bolivia and Brazil), is very exciting and should be made public without delays. It is interesting that the new species is cryptic in wing patterns, and its cryptic nature allowed it to remain unnoticed for over 200 years of research in butterfly taxonomy. Despite the small sample (type series of 22 specimens), we think that our taxonomic conclusions are solid, and genitalic differences are so pronounced that the species status of this taxon is fully justified. However, much remains to be studied, with the most obvious question being the distribution range of *H. intricata*. We hope that our description will stimulate its future studies, including those by citizen scientists and butterfly enthusiasts. Thus we think it is beneficial to describe this new species right away.

Could *H. intricata* be an extreme variation or a subspecies of *H. sosybius*? These two taxa are sympatric and synchronic. They could be found flying together at exactly the same spot, with two individuals of different species landing on the same leaf. We think that prominent and easily observable differences in both male and female genitalia are sufficient to strongly support distinction between *H. intricata* and *H. sosybius* as species under essentially any species concept (De Queiroz 2007). Interestingly, even in the absence of barcode sequences, one can associate sexes of these two species correctly by matching morphology of their genitalia: both sexes of *H. sosybius* possess larger (for specimens of equal size) and less sclerotized genitalia, while both sexes of *H. intricata* are characterized by smaller and more sclerotized genitalia. This morphological match of genitalia suggests that the two butterflies are distinct biological species. Additionally, their DNA barcodes differ by 3.5%, which indicates that the lineages were separated from each other by several million years and should be regarded as distinct evolutionary species.

Could *H. intricata* be a northern subspecies of H. gisella or *H. cucullina*? DNA barcode distance tree (Fig. 66a) shows that *H. intricata* is in the same clade with *H. cucullina* and *H. gisella* (bootstrap support for the clade is about 60%) and is more distant from sympatric *H. sosybius*. Even without the reconstructed tree, analysis of pairwise differences in DNA barcodes (Fig. 66a right, row and column 3 in the matrix) leads to the same conclusion: species closest to *H. intricata* are *H. gisella* (2.2% difference) and *H. cucullina* (2.4% difference). *H. sosybius* appears to be more distant at 3.4% difference. By genitalia, *H. cucullina* is quite different from the other species in its very short, thick and curved penis (Forster 1964, Seraphim et al. 2014). The penis in *H. sosybius* is more gracile. *H. intricata* and *H. gisella* share a shorter, similarly proportioned penis. However, in *H. gisella* the penis is strongly curved (Forster 1964, Seraphim et al. 2014), but in *H. intricata* the penis is bent only slightly, even less than in

H. sosybius. Interestingly, DNA barcodes suggest that *H. cucullina*, despite its radically different penis, might be closer to *H. gisella* (1.7% difference), and *H. intricata* may be more distant from either of these two species (at least 2.2%, Fig. 66). Although it is not clear whether *H. intricata*, *H. gisella* and *H. cucullina* are sympatric in any locality, differences in their genitalia and DNA barcodes argue that they are three biologically distinct species. However, all three form a species group and possibly are a superspecies (Amadon 1966). A comprehensive comparative study of DNA barcodes and male genitalia morphology by Seraphim et al. (2014) suggested a 2% difference in barcodes of *Hermeuptychia* as a sensible indicator of species distinctness. DNA barcodes of *H. intricata* and *H. gisella* reveal a 2.2% difference. This difference coupled with differences in penis shape allow us to comfortably propose *H. intricata* as a new species.

Should the neotype for *H. sosybius* be designated now? It is difficult to derive firm conclusions about taxa without firm identity. We think that it is not prudent to describe a new species sympatric with and hardly separable by wing patterns from *H. sosybius* without clarity about what the name *H. sosybius* stands for. Although the nature of Fabricius types of *H. sosybius* remains unclear and might never come to light, essentially everyone used and uses this name to refer to a common *Hermeuptychia* species widely distributed in the eastern US. Therefore it is sensible to stabilize this meaning by neotype designation. Data we gathered suggests which of the two US species should be *H. sosybius* (in the traditional use of the name) and a specimen of this species was selected as neotype.

What happened to *H. hermes* for USA populations? We agree with Seraphim et al. (2014) that the name *H. hermes* should not be applied to USA *Hermeuptychia* populations. Described from Brazil: Rio de Janeiro (either Ilha Rasa or the city of Rio per G. Lamas, pers. comm.), *H. hermes* is characterized by very distinct genitalia, illustrated by Forster (1964: Abb. 60) and is easily distinguished from other *Hermeuptychia* species by valvae strongly constricted in the middle and a very long saccus (see Seraphim et al. 2014 for a photograph). DNA barcodes of specimens with this genitalia type do not group with *H. sosybius*-like barcodes in trees, and are more similar to *H. maimoune* instead (Fig. 66, Seraphim et al. 2014). *H. hermes* barcodes are over 4% different from any of close to 100 available *Hermeuptychia* barcodes from across the US. For instance, *H. hermybius* barcodes from south Texas are much closer to *H. sosybius* barcodes (2% different) than to *H. hermes* barcodes (4.5%). Even *H. intricata* barcodes are closer to *H. sosybius* barcodes (3.4%) than to *H. hermes* barcodes (4.5%, see Fig. 66). The tree topology (Fig. 66a) is consistent with this distance analysis. As a summary, both male genitalia and barcodes indicate that the name *H. hermes* was incorrectly applied to USA populations. Widespread usage of the name *H. hermes* is simply a consequence of it being the oldest and a tendency to lump butterflies similar in wing patterns. Names that are presently considered junior subjective synonyms of *H. hermes* were proposed on the basis of South American specimens (Lamas 2004). While it will be necessary to clarify the status of these taxa by obtaining DNA barcodes or DNA ID tags from the primary type specimens and by designation of neotypes, it is very unlikely that any of these names could refer to USA *Hermeuptychia* populations. As DNA barcodes

obtained in Seraphim et al. (2014) show, there is hardly any overlap between species at the northern and southern limits of *Hermeuptychia* range. Therefore, *Hermeuptychia* species from Surname is in all likelihood different from a *Hermeuptychia* species in Mexico.

Can *H. hermybius* be identified by wing patterns alone? The *H. hermybius* type series of 101 specimens from all 5 Texas Counties bordering the Rio Grande from Laredo to Brownsville and northeastern Mexico (Tamaulipas & San Luis Potosí) offers excellent material to study variation. This sample suggests that pattern-based identification using the three characters described in the diagnosis above is rather reliable, and *H. hermybius* (in contrast to *H. intricata*) could be mostly identified by wing patterns. Interestingly, while *H. hermybius* is much closer to *H. sosybius* phylogenetically than *H. intricata*, it seems easier to identify by wing patterns. Nevertheless, due to extreme variability of *Hermeuptychia* patterns, some specimens, especially those with smaller, underdeveloped eyespots and other elements of ventral hindwing pattern will not be identifiable by facies.

Are DNA barcode sequences necessary for confident identification of *H. hermybius*? *H. hermybius* is a species very close to *H. sosybius*. Our analysis shows that the best characters to tell between the two species are indeed DNA barcodes (Fig. 66). However, we think that wing-based and male genitalia-based (using measurements and graph in Fig. 63) identification will be unambiguous in most cases without knowing the locality of a specimen. Nevertheless, it is likely that some specimens would not be identifiable with confidence in the absence of DNA barcodes.

Could *H. hermybius* be a southern subspecies of *H. sosybius*? In our opinion, consistency between the differences in DNA barcodes, wing patterns, male genitalia and historic treatment of south Texas populations as a distinct species by several authors (Miller and Brown 1981, brief comment in Neck 1996, Pelham 2008, Warren et al. 2013), although under the incorrect name "*H. hermes*", argues for the species status of this taxon. We see a prominent 2% difference hiatus in DNA barcodes between central Texas and south Texas *Hermeuptychia* populations (Figs 66–67) and very little variation in barcodes of *H. sosybius* across its range from North Carolina to Florida and central Texas. This DNA barcode hiatus correlates with the wing pattern differences: smaller and more uniformly sized eyespots, more wavy brown lines in south Texas populations; and with male genitalia differences that were quantified on a diverse sample (Fig. 63). Finally, without knowing DNA barcodes, experienced butterfly taxonomists who were quite familiar with Satyr butterflies, in particular Miller and Brown (1981), listed USA *Hermeuptychia* populations as two species. However, it remains unknown whether *H. hermybius* and *H. sosybius* are sympatric, and the region between the Lower Rio Grande Valley and central Texas should be explored for possible areas of sympatry.

Is there a specimen age limit for successful DNA barcoding? The oldest specimens we succeeded with were about 120 years old. These were the oldest specimens we tried. We see that DNA fragments into smaller pieces with age. Therefore, for successful amplification, primers for shorter DNA segments should be designed. Per

Materials and methods section, we developed primers for two very short (75 bp and shorter) segments of DNA that contain the highest density of differences between the three US *Hermeuptychia* species. We called these regions ID tags. These tags were successfully and consistently amplified from over 100 years old specimens. Example of the results is shown in Fig. 65. The ID tags allowed us to identify these specimens by DNA and these identifications always matched genitalic identifications. This method could be applied to older specimens and is expected to yield similar success. Potentially, even the oldest preserved butterfly specimen could contain usable DNA that can be extracted and amplified with current methods.

What could be the English name for *H. intricata* and *H. hermybius*? Although some object to using "common" names for butterflies (i.e. the names in the native language of a country the insect inhabits), especially in research publications, we believe that common names are beneficial to attract public interest to butterflies and to disseminate knowledge about them more efficiently, thus possibly aiding their conservation. We suggest "Intricate Satyr" as the English name for *H. intricata* to indicate its delicate wing patterns, cryptic nature, difficulty of identification, and the fact that it has remained overlooked in over 200 years of exploration of North American butterflies despite very significant differences in genitalia. We propose the English name "South Texas Satyr" for *H. hermybius* to emphasize its type locality and distribution in the US, and to highlight the importance of South Texas in the studies of butterfly fauna.

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References

Allen TJ (1997) The butterflies of West Virginia and their caterpillars. University of Pittsburgh Press, 388 pp.

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. Journal of Molecular Biology 215(3): 403–410.

Amadon D (1966) The superspecies concept. Systematic Biology 15(3): 245–249. doi: 10.2307/sysbio/15.3.245

Anken RH (1993) Bemerkungen zu *Hermeuptychia hermes* Fabricius im Südosten Nordamerikas (Lepidoptera: Satyridae). Entomologische Zeitschrift 103(22): 415–419.

Benson DA, Cavanaugh M, Clark K, Karsch-Mizrachi I, Lipman DJ, Ostell J, Sayers EW (2013) GenBank. Nucleic Acids Research 41(Database issue): D36–D42. doi: 10.1093/nar/gks1195

Bickford D, Lohman DJ, Sodhi SH, Ng PKL, Meier R, Winker K, Ingram KK, Das I (2007) Cryptic species as a window on diversity and conservation. Trends in Ecology & Evolution 22(3): 148–155. doi: 10.1016/j.tree.2006.11.004

Brock JP, Kaufman K (2003) Butterflies of North America. Hillstar Editions L. C., New York, 384 pp., Figs, maps.

Calhoun JV (1997) Updated list of the butterflies and skippers of Florida (Lepidoptera: Papilionoidea and Hesperioidea). Holarctic Lepidoptera 4(2): 39–50.

Calhoun JV (2006) A glimpse into a "Flora et Entomologia": The Natural History of the Rarer Lepidopterous Insects of Georgia by J. E. Smith and J. Abbot (1797). Journal of the Lepidopterists' Society 60(1): 1–37.

De Queiroz K (2007) Species concepts and species delimitation. Systematic Biology 56(6): 879–886. doi: 10.1080/10635150701701083

Dereeper A, Guignon V, Blanc G, Audic S, Buffet S, Chevenet F, Dufayard JF, Guindon S, Lefort V, Lescot M, Claverie JM, Gascuel O (2008) Phylogeny.fr: robust phylogenetic analysis for the non-specialist. Nucleic Acids Research 36(Web Server issue): W465–W469.

DeVries PJ (1989) I remember Gordon. Journal of the Lepidopterists' Society 43(4): 343–345.

Fabricius JC (1793) Entomologia systematica emendata et aucta. Secundum classes, ordines, genera, species adjectis synonimis, locis, observationibus, descriptionibus. Hafniae, Christian Gottlieb Proft, Fil. et Soc. 3(1): [vi] + 488 pp.

Field WD (1967) Preliminary revision of butterflies of the genus *Calycopis* Scudder (Lycaenidae: Theclinae). Proceedings of the United States National Museum 119(3552): 1–48. doi: 10.5479/si.00963801.119-3552.1

Forster W (1964) Beiträge zur Kenntnis der Insektenfauna Boliviens XIX. Lepidoptera III. Satyridae. Veröffentlichungen der zoologischen Staatssammlung München 8: 51–188.

Gascuel O (1997) BIONJ: an improved version of the NJ algorithm based on a simple model of sequence data. Molecular Biology and Evolution 14(7): 685–695. doi: 10.1093/oxfordjournals.molbev.a025808

Glassberg J (2007) A Swift guide to the butterflies of Mexico and Central America. Sunstreak Books, Inc., Morristown, [vi] + 266 pp., Figs.

Glassberg J, Minno MC, Calhoun JV (2000) Butterflies through binoculars: a field, finding, and gardening guide to butterflies in Florida. Oxford University Press, Oxford, viii + 242 pp., 44 pls., Figs, 3 tabs, maps.

Grishin NV, Durden CJ (2012) New Bromeliad-feeding Strymon species from Big Bend National Park, Texas, USA and its vicinity (Lycaenidae: Theclinae). Journal of the Lepidopterists' Society 66(2): 81–110.

Hebert PDN, deWaard JR, Landry J-F (2010) DNA barcodes for 1/1000 of the animal kingdom. Biology Letters 6(3): 359–362. doi: 10.1098/rsbl.2009.0848

Howe WH (1975) The butterflies of North America. Doubleday & Company, Inc., New York, xiii + 633 pp., 97 pls., 32 Figs.

ICZN (International Commission on Zoological Nomenclature) (1999) International Code of Zoological Nomenclature, Fourth Edition. International Trust for Zoological Nomenclature, London, xxix + 306 pp. Online version amended "with effect from 1 January 2012" http://www.nhm.ac.uk/hosted-sites/iczn/code/ [accessed January 12, 2014]

Kimura M (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16: 111–120. doi: 10.1007/BF01731581

Lamas G (2004) Satyrinae. In: Heppner JB (Ed) Checklist: Part 4A. Hesperioidea-Papilionoidea. Atlas of Neotropical Lepidoptera. Association for Tropical Lepidoptera/Scientific Publishers, Gainesville, Florida, 220 pp.

Miller LD, Brown FM (1981) A catalogue/checklist of the butterflies of America north of Mexico. Memoir. The Lepidopterists' Society 2: vii + 280 pp.

Murray D, Prowell DP (2004) Molecular phylogenetics and evolutionary history of the neotropical Satyrine Subtribe Euptychiina (Nymphalidae: Satyrinae). Molecular Phylogenetics and Evolution 34(1): 67–80. doi: 10.1007/BF01731581

Neck R (1996) A field guide to butterflies of Texas. Gulf Publishing Company, Houston, Texas, xvii + 323 pp.

Nicolay SS (1989) Obituary: Gordon Burgess Small, Jr. (1934. 1989). Journal of the Lepidopterists' Society 43(4): 340–343.

Opler PA, Krizek GO (1984) Butterflies east of the Great Plains. An illustrated natural history. The Johns Hopkins University Press, Baltimore and London, xvii + 294 pp., 54 pls., 38 Figs, 11 tabs.

Opler PA, Lotts K, Naberhaus T (coordinators) (2013) Butterflies and moths of North America. http://www.butterfliesandmoths.org/ (Version 10052013)

Opler PA, Malikul V (1992) A field guide to eastern butterflies. Houghton Mifflin Company, Boston, xix + 396 pp., 48 pls., 7 Figs, maps.

Opler PA, Warren AD (2002) Butterflies of North America. 2. Scientific names list for butterfly species of North America, north of Mexico. Colorado State University, C. P. Gillette Museum of Arthropod Diversity, Fort Collins, [2] + 79 pp.

Pelham JP (2008) A catalogue of the butterflies of the United States and Canada with a complete bibliography of the descriptive and systematic literature. Journal of Research on the Lepidoptera 40: i-xiii, 1–652.

Peña C, Nylin S, Freitas AVL, Wahlberg N (2010) Biogeographic history of the butterfly subtribe Euptychiina (Lepidoptera, Nymphalidae, Satyrinae). Zoologica Scripta 39: 243–258. doi: 10.1111/j.1463-6409.2010.00421.x

Robbins RK (1991) Evolution, comparative morphology, and identification of the Eumaeine butterfly genus *Rekoa* Kaye (Lycaenidae: Theclinae). Smithsonian Contributions to Zoology 498, 64 pp.

Robbins RK, Lamas G (2006) Stabilizing the nomenclature of fabrician names of North American hairstreaks (Lycaenidae: Theclinae: Eumaeini). Journal of the Lepidopterists' Society 60(2): 86–91.

Scott JA (1986) The butterflies of North America. A natural history and field guide. Stanford University Press, Stanford, xv + 583 pp., 64 pls., 71 Figs, 2 tabs, maps.

Seraphim N, Marín MA, Freitas AVL, Silva-Brandão KL (2014) Morphological and molecular marker contributions to disentangling the cryptic *Hermeuptychia hermes* species complex (Nymphalidae: Satyrinae: Euptychiina). Molecular Ecology Resources 14(1): 39–49. doi: 10.1111/1755-0998.12161

Silva-Brandão KL, Lyra ML, Santos TV, Seraphim N, Albernaz KC, Pavinato VAC, Martinelli S, Cônsoli FL, Omoto C (2011) Exploitation of mitochondrial nad6 as a complementary marker for studying population variability in Lepidoptera. Genetics and Molecular Biology 34(4): 719–725. doi: 10.1590/S1415-47572011000400028

Smith AZ (1986) A history of the Hope Entomological Collections in the University Museum, Oxford with lists of Archives and Collections. Clarendon Press, Oxford, xiii + 172 pp.

Steinhauser SR (1981) A revision of the *proteus* group of the genus *Urbanus* Hübner. Lepidoptera: Hesperiidae. Bulletin of the Allyn Museum 62: 1–41.

Tveten JL, Tveten GA (1996) Butterflies of Houston and Southeast Texas. University of Texas Press, Austin, x + 292pp.

Vane-Wright RI (2010) William Jones of Chelsea (1745-1818), and the need for a digital, online 'Icones'. Antenna 34(1): 16–21.

Warren AD, Davis KJ, Stangeland EM, Pelham JP, Grishin NV (2013) Illustrated Lists of American Butterflies. http://www.butterfliesofamerica.com [accessed 30.XII.2013]

Zakharov EV, Lobo NF, Nowak C, Hellmann JJ (2009) Introgression as a likely cause of mtDNA paraphyly in two allopatric skippers (Lepidoptera: Hesperiidae). Heredity (Edinburgh) 102(6): 590–599.

Zimsen E (1964) The type material of I. C. Fabricius. Copenhagen, Munksgaard, 656 pp., 3 Figs.

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A new cryptic *Sympistis* from eastern North America revealed by novel larval phenotype and host plant association (Lepidoptera, Noctuidae, Oncocnemidinae)

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Abstract

A *Triosteum*-feeding species of *Sympistis* is described from eastern North America: *Sympistis forbesi* **sp. n.** Identity of the new species is most reliably determined from larval morphology and host plant association—both adult scaling and genitalic characters overlap with those of *Sympisitis chionanthi*, a *Chionanthus* and *Fraxinus* feeder.

Keywords

Cryptic species, *Triosteum*, Caprifoliaceae, iridoid glycosides, unequal evolutionary rates

Introduction

Sympistis Hübner is the second largest genus of North American macrolepidopterans, with 176 recognized species (Troubridge 2008, Lafontaine and Schmidt 2010) and many others awaiting formal description. *Sympistis forbesi* sp. n. was first mentioned as a *Triosteum*-feeding variant of *Adita chionanthi* (J. E. Smith) by Rummel (1921)

who published a brief account of the larva and its biology. Although Forbes (1954) treated the *Triosteum*-feeder as a "well-marked food strain" of *Adita chionanthi* [now *Sympistis chionanthi*], it is clear that he suspected that the moth represented a valid species, because he provided differential diagnoses for both the adult: "a little less crispy marked, the anal dash a little diffuse, or located in a blackish smudge," and the last instar: "head green, shaded behind with pale brownish, body yellow-green, the dorsum largely purple-red, with a paler often greenish dorsal line, and a fine white subdorsal near edge of the purple portion; tubercles i and ii small and white, on it. Three dark green lateral lines, the ground usually darkened between the two lower; a broad whitish stigmata line." Larvae of the two species are figured in Wagner et al. (2011). *S. chionanthi* feeds on *Fraxinus* L., *Chionanthus* L., and perhaps other members of the Oleaceae, whereas *S. forbesi* is believed to be associated only with *Triosteum* L. in the Caprifoliaceae, although larvae can be reared on *Fraxinus* in the laboratory. Given the obvious differences in larval coloration and diet, it is evident that Forbes, and perhaps other noctuoid workers of his day, placed less weight on larval and life history characters than lepidopterists afford them today.

Our motivation for the description of the *Triosteum*-feeding *Sympistis* is that both *S. chionanthi* and *S. forbesi* are worthy, or are likely to become, conservation targets. *S. forbesi* is believed to be extirpated from New Jersey (where Rummel first reported the species) and no extant colonies are known in New York (where Forbes and other Cornell lepidopterists knew it). We think it likely that it is declining or already extinct from much of its former eastern range due to decline in the abundance of *Triosteum* (feverwort), which fared better in the open agricultural landscapes of the two previous centuries. Increased grazing pressure by white-tailed deer is also thought to be a threat to the host plant and its herbivore fauna (Wagner et al. 2011). The only known extant colonies are those present in prairie areas where *Triosteum* is still locally common. *S. chionanthi*, while still widespread across its range (extending from North Dakota to Nova Scotia south to at least Virginia and Kansas), is threatened by the destruction of its primary host by the Emerald Ash Borer (*Agrilus planipennis* Fairmaire) (listed in Wagner 2007).

Below we describe the new *Sympistis*, illustrate the larval, pupal, and adult stages and provide a brief account of the biology of the new species.

Methods

The adult description of *S. forbesi* is based on 45 pinned specimens from Iowa, Illinois and Minnesota. Seventy-one specimens of *S. chionanthi* from Connecticut and New York were studied (n=71). The larval description of *S. forbesi* is based on 15 preserved larvae and 65 larval images (GGC, ISIC, UCMS). Larvae were compared to 7 preserved larvae and 11 larval images of *S. chionanthi* (CUIC, GGC, NYSM, UCMS). Genitalia of the male type and one female paratype were prepared and mounted according to Lafontaine (2004) except that the preparations were mounted in euparol.

Two additional genitalic preparations were left in glycerin. Six slide mounted genitalic preparations made by John G. Franclemont, identified as *Triosteum*-feeding strains of *S. chionanthi*, were borrowed and examined from CUIC. Thirteen *S. chionanthi* genitalic preparations (from New York, Connecticut, Manitoba, Ontario, and Saskatchewan) were examined. COI sequences were generated by the Barcodes of Life Project. Sequences for two *S. forbesi* specimens (Barcodes of Life Project Numbers CNCLEP 81921 and CNCLEP 81922) and six *S. chionanthi* from Ontario and Quebec (Barcodes of Life Project Numbers CNCLEP 81919, CNCNoctuoidea 7959, DH007094, DH009854, 2005-ONT-1897, 2005-ONT-1928) have been deposited at GenBank.

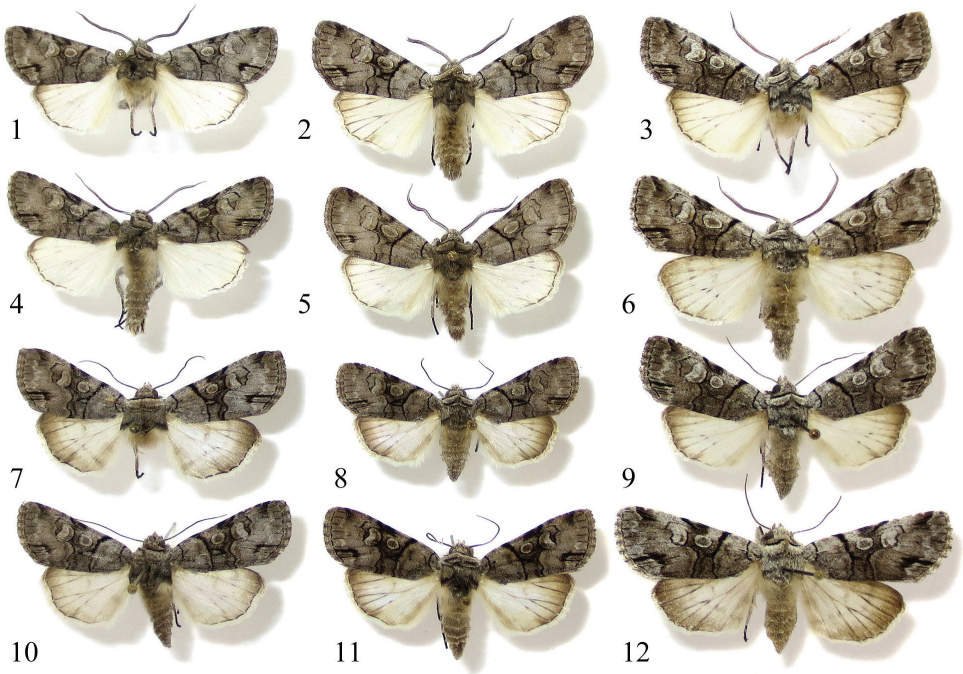
Abbreviations

CNC	Canadian National Collection, Ottawa, Ontario, Canada.
CUIC	Cornell University Insect Collection, Ithaca, New York, USA
GGC	George Godfrey Collection, Athens, IL, USA
CHC	Chuck Harp Collection, Littleton, Colorado, USA
ISIC	Iowa State Insect Collection, Iowa, USA
NDSU	North Dakota State University, North Dakota, USA
NMNH	National Museum of Natural History, Washington D.C., USA
NYSM	New York State Museum, Albany, New York, USA
UCMS	University of Connecticut, Storrs, Connecticut, USA

Taxonomy

***Sympistis forbesi* Zacharczenko & Wagner, sp. n.**
http://zoobank.org/A3005B5D-DCC6-42D1-B743-E1CD7A843763
http://species-id.net/wiki/Sympistis_forbesi
Figs 1, 2, 4, 5, 7, 8, 10, 11, 13, 14, 18–32, 34–36

Material examined. HOLOTYPE male (Fig. 1) IA: Boone Co., Little Bluestem Prairie [41°53'52N, 93°52'10"W], [larva] 29 May 2010, Mary Jane Hatfield, 051B-B10, [adult emerged] 9 September 2010, host: *Triosteum perfoliatum*; Genitalia CNC slide # ♂ 16516; Barcodes of Life Project # CNCLEP 81921, leg removed, DNA extracted. Deposited at UCMS, Storrs, Connecticut, USA. **Paratypes (adults).** (22 males, 23 females) **Iowa:** Polk Co., Snyder Farm [41°46'23.51"N, 93°29'21.38"W] [larva] 316 May 2009, Mary Jane Hatfield; DLW Lot: 2010E96, emerg: 29 August 2010, Host: *Triosteum perfoliatum*, (1 ♂) (UCMS); Polk Co., Snyder Farm [41°46'23.51"N, 93°29'21.38"W] [larva] 30 May 2009, Mary Jane Hatfield, emerg: fall 2009, Host: *Triosteum perfoliatum*, (1 ♀) (UCMS);Polk Co., Snyder Farm [41°46'23.51"N, 93°29'21.38"W], 9 May 2010 [larva], 004-P10, Mary Jane Hatfield, [adult] found



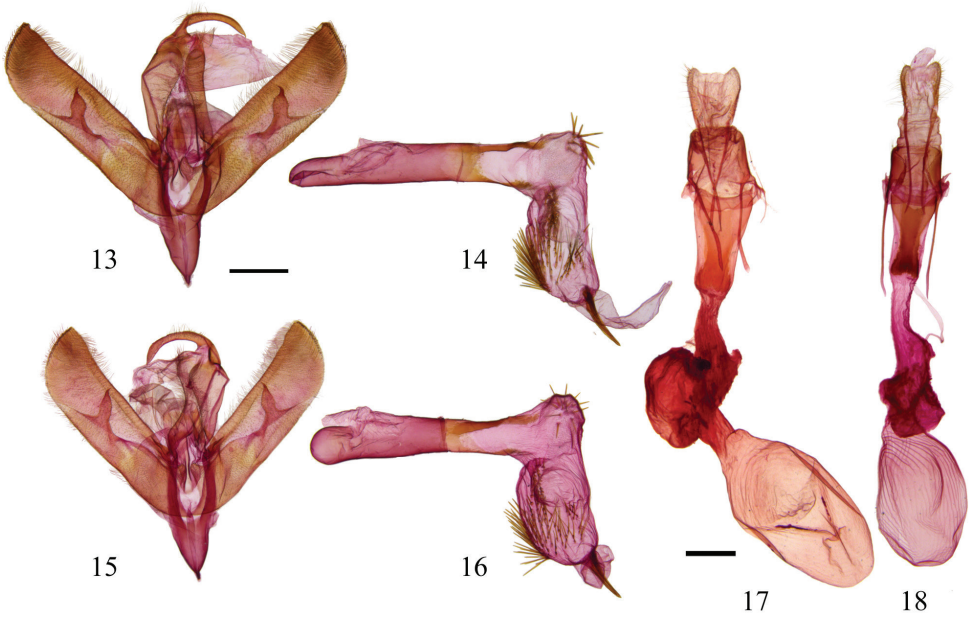
Figures 1–12. Adults of *S. forbesi* and *S. chionanthi.* **1** ♂ *S. forbesi* HOLOTYPE, IA: Boone Co., Little Blue Stem Prairie, ex larva on *Triosteum* (UCMS) **2** ♂ *S. forbesi,* IL: Champaign Co., Mahomet, ex larva on *Triosteum* (CUIC) **3** ♂ *S. chionanthi,* NY: Tompkins Co., Ithaca, ex ova, reared on *Fraxinus* (CUIC) **4** ♂ *S. forbesi*, IA: Boone Co., Little Blue Stem Prairie, ex larva on *Triosteum* (UCMS) **5** ♂ *S. forbesi,* IL: Champaign Co., Mahomet, ex larva on *Triosteum* (CUIC) **6** ♂ *S. chionanthi,* CT: Windham Co., Hampton, adult at light (UCMS) **7** ♀ *S. forbesi*, IA: Boone Co., Little Blue Stem Prairie, ex larva on *Triosteum* (UCMS) **8** ♀ *S. forbesi,* IL: Champaign Co., Mahomet, ex larva on *Triosteum* (CUIC) **9** ♀ *S. chionanthi,* NY: Tompkins Co., Ithaca, ex ova, reared on *Fraxinus* (CUIC) **10** ♀ *S. forbesi*, IA: Polk Co., ex larva on *Triosteum* (UCMS) **11** ♀ *S. forbesi,* IL: Champaign Co., Mahomet, ex larva on *Triosteum* (CUIC) **12** ♀ *S. chionanthi,* CT: Windham Co., Pomfret, adult at light (UCMS).

dead 9 September 2010, Host: *Triosteum perfoliatum* (1 ♂) (UCMS); Boone Co., Little Bluestem Prairie [41°53'52N 93°52'10"W], May 2010 [larva], Mary Jane Hatfield, 051-D-B10, emerged 19 September 2010, Host: *Triosteum perfoliatum* (1 ♀) (UCMS); Boone Co., Little Bluestem Prairie [41°53'52N 93°52'10"W], 29 May 2010 [larva], Mary Jane Hatfield, 051C-B10, 13 September 2010 [emerged], Host: *Triosteum perfoliatum* (1 ♂) (UCMS); Boone Co., Little Bluestem Prairie [41°53'52N 93°52'10"W], 29 May 2009 [larva], Mary Jane Hatfield, 051B-B10, emerged 11 September 2010, Host: *Triosteum perfoliatum;* Barcodes of Life Project # CNCLEP 81922, leg removed, DNA extracted; Genitalia Slide CNC #16517 ♀ (UCMS); Boone Co., Little Bluestem Prairie, 41°53'53.83"N, 93°52'10.31"W, Sept. 2011, MJ Hatfield coll. (3 ♂) (2 UCMS, 1 ISIC); Boone Co., Little Bluestem Prairie, 41°53'53.83"N, 93°52'10.31"W, prairie remnant edge, larva May 5 2013, MJ Hatfield coll., 010E-B13 (3 ♀) (1 UCMS,

2 ISIC); Boone Co., Little Bluestem Prairie, 41°53'53.83", N 93°52'10.31"W, prairie remnant edge, larva May 5 2013, MJ Hatfield coll., 010C-1-13 (1 ♂) (ISIC); Kossuth Co., Algona, larva 20 May–14 June 2013, emerged 5 Sept. 2013, Matt Kenne coll. (1 ♀) (UCMS); Kossuth Co., Algona, larva 20 May–14 June 2013, emerged 10 Sept. 2013, Matt Kenne coll. (1 ♀) (UCMS); Kossuth Co., Algona, larva 20 May–14 June 2013, emerged 30 August 2013, Matt Kenne coll. (2 ♀) (UCMS); Kossuth Co., Algona, larva 20 May–14 June 2013, emerged 2 Sept. 2013, Matt Kenne coll. (1 ♂) (ISIC); Kossuth Co., Algona, larva 20 May–14 June 2013, emerged 3 Sept. 2013, Matt Kenne coll. (1 ♂) (NDSU); Kossuth Co., Algona, larva 20 May–14 June 2013, emerged 3 Sept. 2013, Matt Kenne coll. (1 ♀) (NDSU); Kossuth Co., Algona, larva 20 May–14 June 2013, emerged 6 Sept. 2013, Matt Kenne coll. (1 ♀) (ISIC); **Illinois:** Champaign Co., Mahomet, reared ex larva, 9–14 Oct. 1976, 27 Aug. 1976, 13 Aug. 1981, G. Godfrey coll. (7 ♂, 5 ♀) (CUIC); Cook Co., Elk Grove, [adults] bred 20–24 Aug. 1941 and 26–28 Aug. 1942, A.K. Wyatt (1 ♂, 3 ♀) (CUIC); **Minnesota:** Houston Co., Perkin's Bluff Prairie, 43°47'8.85"N, 91°36'58.63"W, larva 11 May 2013, emerged 5 Sept. 2013, Mary Jane Hatfield coll. (3 ♀) (1 CNC, 1 CHC, 1 NMNH); Houston Co., Perkin's Bluff Prairie, 43°47'8.85"N, 91°36'58.63"W, larva 11 May 2013, emerged 5 Sept. 2013, Mary Jane Hatfield coll. (1 ♂) (NMNH); Houston Co., Perkin's Bluff Prairie, 43°47'8.85"N, 91°36'58.63"W, larva 11 May 2013, Mary Jane Hatfield coll. (4 ♂) (1 CNC, 1 CHC, 2 UCMS). **Paratypes (larvae). Iowa:** Polk Co., Snyder Farm [41°46'23.51"N, 93°29'21.38"W], Col: 29 March 2012 [larva], 9 May 2012 [preserved]; Mary Jane Hatfield, Host: *Triosteum perfoliatum* (UCMS); Story Co., Harker Savannah [41°54'6.73"N, 93°30'31.21"W], Col: 29 April 2012 [larva], 9 May 2012, [preserved]Mary Jane Hatfield, Host: *Triosteum perfoliatum* (UCMS); Story Co., Harker Savannah [41°54'6.73"N, 93°30'31.21"W], Col: 29 April 2012 [larva], 9 May 2012 [preserved]; Mary Jane Hatfield, Host: *Triosteum perfoliatum* (UCMS); Winneshiek Co., [43°27'51.96"N, 91°38'15.73"W], Col: 15 May 2012 [larva], 18 May 2012 [preserved], Mark Leoschke, Host: *Triosteum perfoliatum* (UCMS); Allamakee Co., [43°25'16.65"N, 91°16'54.53"W], Col: 23 May 2012 [larva], 24 May 2012 [preserved], Mark Leoschke, Host: *Triosteum perfoliatum* (UCMS); Boone Co., Danielle Wirth property (oak savannah) [41°52'11.29"N, 93°52'55.10"W], Col: 22 May 2010 [larva], 28 May 2010 [preserved], Mary Jane Hatfield, Host: *Triosteum perfoliatum* [dissected] (UCMS).

Etymology. We name the species after William T. Forbes, North America's premier lepidopterist over a 40-year period from 1920 to 1960. Forbes' understanding of the species and higher-level taxonomy of eastern Macrolepidoptera was extraordinary, with the vast majority of his taxonomic decisions standing the test of time (and additional data). His four-volume treatise on the *Lepidoptera of New York and Neighboring States* remains the definitive work on eastern moths, especially for most Microlepidoptera.

Diagnosis. Adult. *Sympistis forbesi* averages slightly smaller than *S. chionanthi*. The scales over the thorax are smaller, more densely packed. In most individuals there are fewer white scales on the thorax and forewing: e.g., the costal margin, wing base, and orbicular and reniform spots have fewer white scales than most individuals of



Figures 13–18. *Sympistis forbesi* and *S. chionanthi* genitalia **13** *Sympistis forbesi* HOLOTYPE male**,** IOWA: Boone Co., Little Blue Stem Prairie, Genitalia CNC slide # 16516 ♂; scale = 1mm **14** aedoeagus, same data **15** *S. chionanthi* male, MANITOBA, Cartwright, Genitalia CNC slide # 16515 ♂ **16** aedoeagus, same data **17** *S. chionanthi* female, SASKATCHEWAN, 8 mi NW Stewart, 1800', Genitalia CNC slide # 13192 ♀; scale = 1mm **18** *Sympistis forbesi* paratype female, same data as male, Genitalia CNC slide # 16517 ♀.

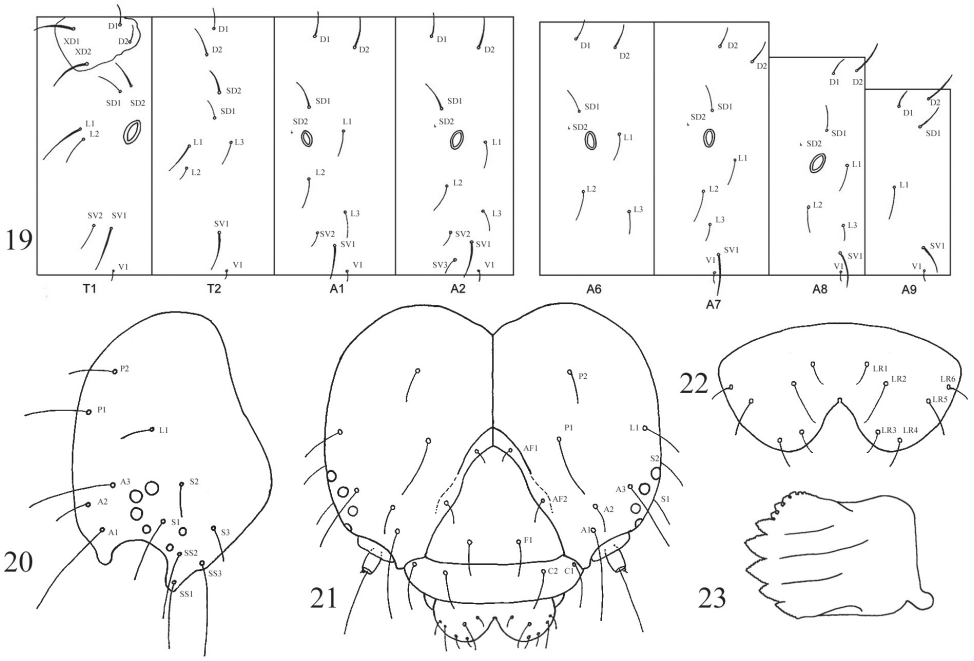
S. chionanthi. Additionally, the anal dash is often more J-shaped and the fringe is only faintly checkered, lacking the pure white scales seen in many *S. chionanthi*. In the hindwing, there is a more distinctive terminal line and the apex tends to have more black scales extending onto the fringe. The rami of male antennae through the basal half of the antenna average 0.50–0.65 mm in *S. forbesi,* and 0.55–0.70 mm in *S. chionanthi.* **Larva**. The larva provides unambiguous morphological characters that allow recognition of this new species. The last instar is mostly green with a reddish dorsum (red coloration is added through mid to late instars); there are no black or brown markings as in *S. chionanthi* (in particular, the black subdorsal stripe characteristic of *S. chionanthi* is absent from all instars of *S. forbesi*). Body smaller, more elongate and modestly tapered at both ends, especially relative to the robust habitus of *S. chionanthi*. Head width of ultimate instar of *S. forbesi* 2.5–2.8 mm; head width of *S. chionanthi* 3.0–3.2 mm. Spiracular height consistently smaller in *S. forbesi* compared to *S. chionanthi*–mean spiracular heights of A1–A6 are 0.30 and 0.36 mm, respectively. Mean crochet number of *S. forbesi* on A3–A6 and A10 are 17, 19, 20, 21, and 20; mean crochet number of *S. chionanthi* on A3–A6 and A10 are 27, 28, 32, 32, and 33.

Description of adult. Male. Forewing length: 14.5–16 mm (n=23, reared from wild larvae). Ground color warm gray. **Head.** Antenna biramous; rami approximately

0.50–0.65 mm through basal half of antenna. Forward-facing tuft of scales just above faint black line between eyes. **Thorax.** Gray, medial prothoracic tuft, edged with black, preceded by conspicuous transverse black line. Black edging of tuft continues laterad to wing base. Tegula steely gray, indistinct thick band of dark scales at back. Legs with mix of dark and light scales. Tarsi dark brown or black. **Forewing.** Thin, smoothly curved basal and antemedial lines. Thickened antemedial line tapering to inner margin. Orbicular spot gray centrally and pale gray peripherally, thinly edged with black. Medial line ill defined; field proximal to reniform spot with numerous dark scales, forming two dark fascia along costa above orbicular and reniform spots. Black line or open triangle in position of claviform spot. Postmedial line running parallel to medial line, connecting to base of reniform and looping around toward margin, finally connecting to dark fascia along costa. Anal dash usually crisp, occasionally absent, subtended by sharp or diffuse black spot basad, forming J-shape. Subterminal line forming black fascia at costa, but otherwise pale gray, weakly developed to nearly obsolescent. Fringe weakly checkered, without white scaling. **Hindwing.** Pearly white with thin, crisp terminal line except at apex where diffuse field of black scales extends through fringe. Postmedial line obsolescent in males. **Abdomen.** Mixture of light and dark scales and hairs. Whitish scales along posterior margin of pregenital abdominal terga. **Male genitalia.** Valves elongate, nearly parallel sided with flat-topped projection from apex; bulbous clasper with claw-like apex that curves mesad; corona of fine setae of variable lengths. Juxta poorly differentiated. Uncas curved, gradually tapering, apex drawn into fine, curved spine. Saccus V-shaped, drawn into point anteriorad. Aedeagus cylindrical, variously sclerotized with vesica bearing approximately one dozen spines on elbow-bend and numerous longer, narrower spines over bulbous subapical region; terminus armed with single stout spine nearly 1 mm in length (as large as uncus).

Female. Forewing length: 14–16.5 mm (n=10, reared from wild larvae). Similar to male, but with substantially more fuscous scaling in submarginal region of hindwing; often with faint postmedial band. Antenna simple, without rami. **Female genitalia.** Posterior and anterior apophyses slender, elongate, ca. 2.5 × length of sclerotized portion of A8; lamella antevaginalis sclerotized, winged anteriorally and posteriorally, with posterior part about ostium bursae cleft and thus appearing somewhat flipper-like; anterior end less flared, ca. ½ width and only shallowly cleft, and more strongly sclerotized. Appendix bursae well developed; ovate corpus bursae ca. 2 × size of appendix bursae with parallel thickenings most evident posteriorad.

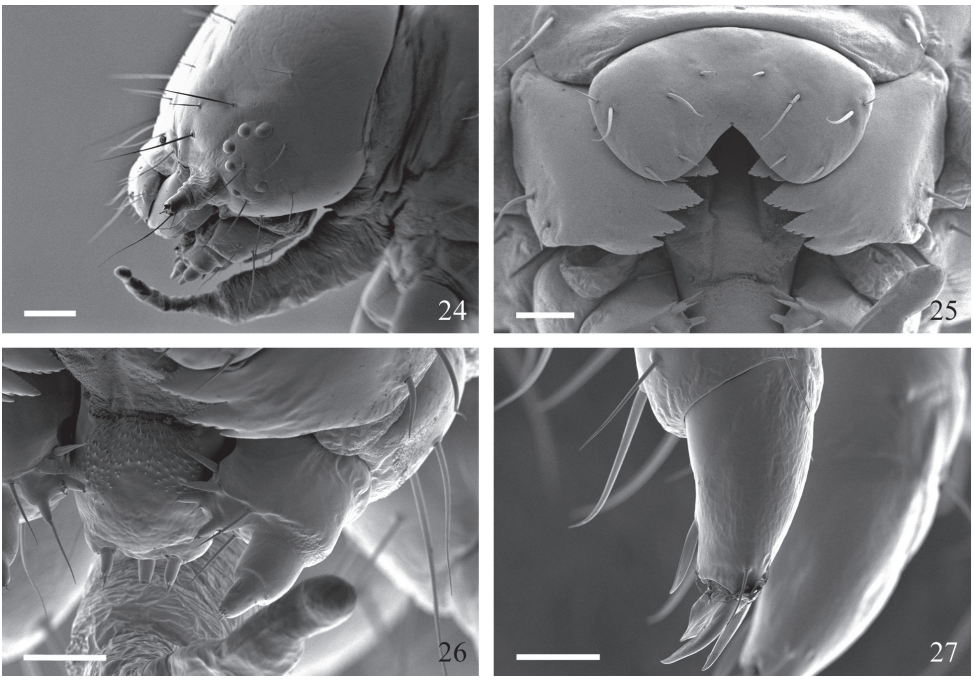
Description of pupa (Figs 28, 29). 16–19 mm long, 4.4–5.0 mm wide. Orange brown to deep chestnut brown, mostly smooth except for deeply pitted anterior portion of abdominal segments A4–A7. Primary setae extremely short, difficult to locate. Labial palpus visible, subequal to visible portion of profemur. Foreleg with cuminate apex, ending in abrupt spine. Proboscis extending just beyond antenna and midleg, nearly reaching end of wing. Labrum roughly shovel shaped with truncated apex. Eyepiece and frons ornamented with dense micro-ridging. Spiracular scars elongate, five times longer than wide. Cremaster ending in pair of minute thorn-like spines; cremaster deeply wrinkled and heavily sclerotized at base.



Figures 19–23. *Sympistis forbesi* larva. **19** chaetotaxy **20** head, lateral **21** head, frontal **22** labrum **23** mandible.

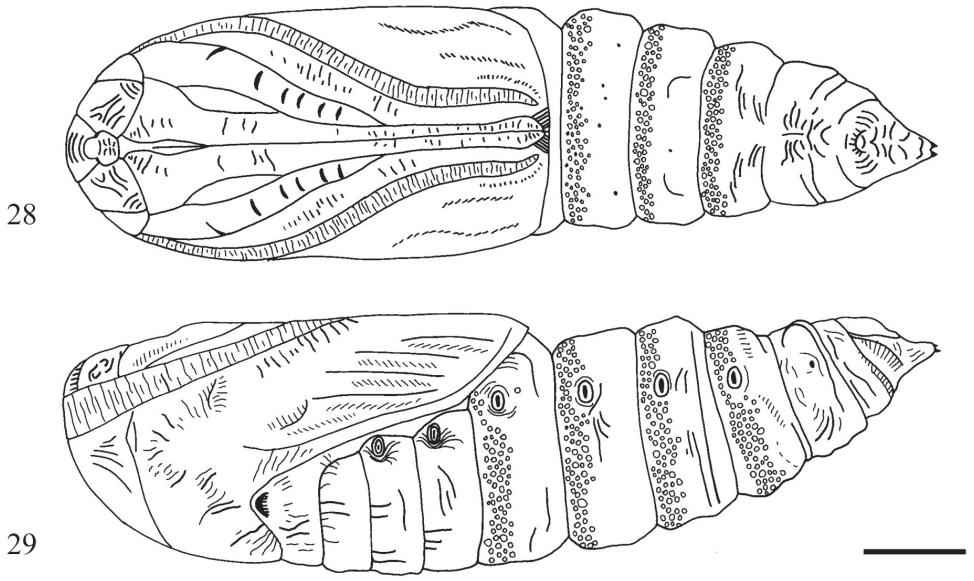
Description of living final instar (Figs 31, 32). Ground color sea to mint green with pink to red dorsum and pale longitudinal striping along sides of trunk; A8 modestly humped. Reddish dorsum composed of pink to pale red middorsal stripe flanked by darker red addorsal stripes; dorsal pinacula white. Mostly broken white pinstripe zigzags through D1 pinacula. Red dorsal area bounded by pale (green to white) subdorsal pinstripe. Two supraspiracular pinstripes edged below with darker green. Lateral stripe, greenish white, roughly equal to height of spiracles, extending along lower end of spiracles. Prolegs on A3 and A4 about half size of those on A5 and A6. Head pale to dark brown above, often with pink to reddish flush; labrum greenish white, shallowly rugose frons, gena with three whitish lines that anastamose about stemmata.

Description of living early instars (Fig. 30). All instars elongate, smooth, with numerous stripes; A8 modestly humped. First and second instars reddish brown and white, shiny; middorsal white stripe enlarged over anterior half of A8. Subspiracular white stripe thickened, enlarged to include each spiracle along trunk. All pinacula distinct, raised, brown black. First instar head width 0.15–0.16 mm. Second instar head width 0.40–0.56 mm. Third instar green and white, with brown-black pinacula; head width 0.90–1.00 mm. Fourth instar with small white pinacula as in final instar; head width 1.50–1.80 mm.

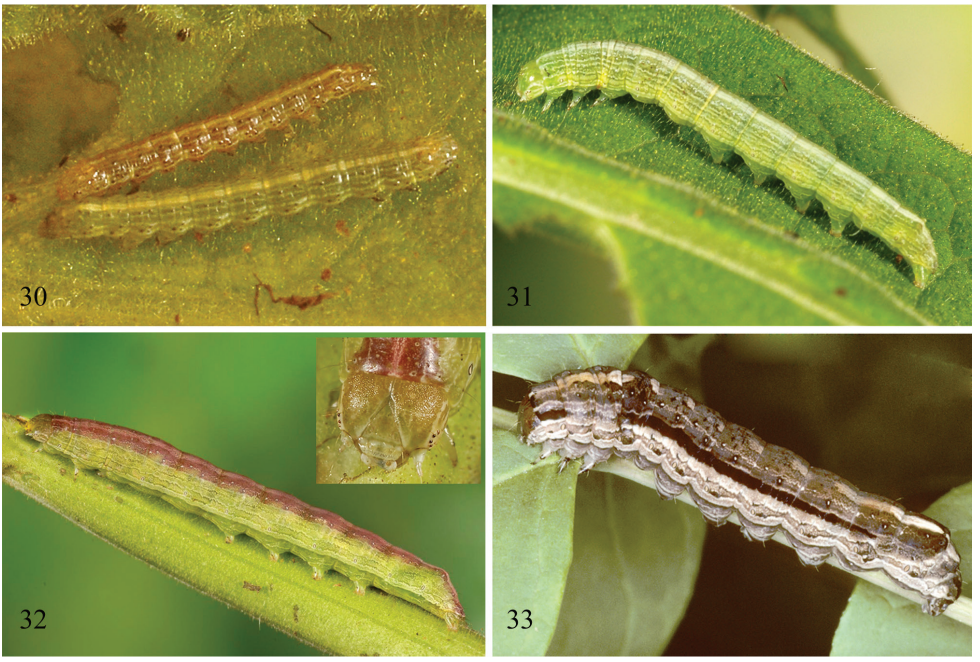


Figures 24–27. *Sympistis forbesi* middle instar. **24** head, lateral, with adenosma extruded; scale = 250 μm **25** labrum, mandibles, and oral cavity; scale = 100 μm **26** hypophryngeal complex (center left) and maxilla (center right); scale = 100 μm **27** prothoracic leg (note apical and subapical blade-like setae proximal to claws); scale = 100 μm.

Description of preserved final instar (Figs 19–27). **Head.** Texture microtuberculate. Width 2.5–2.8 mm. Field of brown aggregated spots on vertex, especially between P setae and L1. Second group of spots caudad of S3. P1 2 × length of P2. A1 longest seta on head (Figs 20, 21, 24). V-shaped medial cleft about 2/5 labral depth (Figs 22, 25). Spinneret short, subequal to labial palpus (Fig. 26). Mandibles simple, inner surface mostly smooth (Fig. 23). **Body.** Length 34–44 mm. Integument smooth; lightly sclerotized prothoracic shield and anal plate. Primary setae short, most approximately 2 × height of spiracle on same segment (Fig. 19). **Thorax.** SV1 longest seta on thoracic segments, ca. 3 × height of prothoracic spiracle and 1½ × height of SV2; spiracular height: 0.30–0.32 mm. Prothorax with SD1 and SD2 free from shield, positioned above spiracle. L1 3 × longer than L2 on T2 and T3. Meso- and metathorax with D and SD setae more or less vertically aligned (Fig. 19). Thoracic legs with apical and subapical blade-like setae proximal to claws (Fig. 27). **Abdomen.** Two SV on A1, three SV on A2. L1 directly behind spiracle on A1–A6 and A8, displaced ventrad on A7. D2 becoming increasingly procumbent towards caudal end of body (Fig. 19). D2 seta on A8 and A9 arising from slightly elevated and pigmented, rearward-facing wart. A10 with D and SD setae on rearward facing warts. Spiracular height of A1 through



Figures 28–29. *Sympistis forbesi* pupa **28** ventral **29** lateral.



Figures 30–33. *Sympistis forbesi* and *S. chionanthi* larvae. **30** *Sympistis forbesi* second (upper) and third (lower) instars. IA: Boone Co., Little Blue Stem Prairie, May 2011, ex *Triosteum perfoliatum* **31** *Sympistis forbesi* middle instar, same collection data **32** *Sympistis forbesi* mature last instar, same collection data **33** *S. chionanthi* mature last instar, NY: Albany Co., Albany, female fall 1995, ex ova reared on *Fraxinus americana*, DLW Lot: 1996F32.

A6 0.28–0.32 mm; height on A7 0.26–0.28 mm; height on A8 0.32–0.35 mm. Crochet numbers on A3–A6 and A10 as follows: 16–18, 17–22, 19–22, 20–23, and 20.

Remarks. We were unable to identify any consistent genitalic differences in either the male or female genitalia that distinguish *S. forbesi* from *S. chionanthi*. Our type series is based on reared material, as we know of no definitive structural or patterning characters that will assure certain identification of light-collected adults. Hence, we caution that features discussed in the diagnosis and description may be attributes more typical of reared (unflown) specimens. For example the slightly smaller size and darker coloration that we note above for *S. forbesi* could be rearing artifacts.

Distribution. Locally common in Midwest, especially prairies. Most commonly found in Iowa, Illinois, and Minnesota. Believed to be extirpated from eastern portion of range in New York and New Jersey. Given that the genus *Triosteum* occurs from southern Canada to Texas and eastward, it is probable that the range of the new species is more extensive than circumscribed here.

Biology. So far as known larvae are specialists on members of the genus *Triosteum*, also known as horse-gentian or feverwort, of the family Caprifoliaceae. Nearly all our larval collections are from *T. perfoliatum* (feverwort). We found a few larvae of what appeared to be the same species on *T. aurantiacum* in Iowa. In the laboratory, larvae from *T. perfoliatum* readily accepted and matured on *T. aurantiacum*. In two separate instances, larvae were successfully reared to pupation on *Fraxinus* as well, a widely used host plant of *S. chionanthi. S. forbesi* larvae grew more slowly on *Fraxinus*, and maintained their typical green and pink coloration (MJH unpublished data, M. Keene personal communication). *S. forbesi* is univoltine with a single generation that emerges, flies, and mates in late summer, mostly in early September. Females presumably lay eggs on or near the stems of *Triosteum*. Above-ground tissues of the host die and senesce over the winter. MJH has found first instars on unopened leaves that were just pushing forth from the ground in early March (in Iowa). Larvae complete their development by mid-June. Early instars feed exclusively on new leaves, principally of the apical meristem, before the leaves have had a chance to open and expand to full size. Where the moth is common and when collections are made through the first half the season, partially opened leaf fascicles often yield larvae that were not seen at the time of collection. Last instars also consume new leaves, but are content to feed on fully expanded leaves and flowers (Figs 34–36). All instars are cryptic in both color and habit. The late instars rest along a shoot head down, often near flowers, where their coloration is well matched to that of the stem and reddish-pink *Triosteum* petals and sepals. Densities can be high with more than a dozen larvae on a single shoot; on several occasions we noted cases where the larvae of *S. forbesi* severely damaged the apical portions of their host plant. Prepupae form a slight cocoon below ground; the summer months are passed as a pupa.

Barcoding. In a neighbor-joining tree based on J. D. Lafontaine's unpublished barcodes for 137 North American oncocnemidine noctuids (representing 687 individuals), *S. chionanthi* (n=7; CT, Quebec, Ontario, Alberta) and the new species (n=2; both Iowa) grouped together in a "cluster" separate from other North American

Figures 34–36. *Sympistis forbesi* IA: Boone Co., Little Blue Stem Prairie, May 2011 on *Triosteum perfoliatum* **34** three larvae secreted in a leaf axil; note frass accumulation **35** larvae on new spring leaves; note two larvae on new leaf bundle and one on foreground leaf **36** last instar on a flower of *Triosteum perfoliatum,* matching the color of the flower and petioles.

Sympistis, and each taxon was reciprocally "monophyletic," although the two groups differed by less than 1% from one another. In a second analysis, focused on "*Adita*-group" *Sympistis* that included 13 individuals from across North America, again the two *Triosteum* feeders grouped in their own cluster.

Discussion. *S. chionanthi* was described by J. E. Smith in Abbot and Smith 1797 based on a painting of the adult, caterpillar, pupa, and the host *Chionanthus virginica* Linn. (fringetree) (family Oleaceae) by Abbot. As is the case for all of taxa drawn by Abbot, there is no type specimen for *S. chionanthi.* The common name "Grey O Moth" was given for the "O" shaped orbicular spot on each forewing. Abbot's rendering of the larva depicts a robust caterpillar that is pale brown laterally, shaded with darker brown dorsally, and bears a thick black subdorsal stripe, a slight stripe behind the head and a moderately humped A8 segment. His illustration is undoubtedly a match for the current-day *S. chionanthi* caterpillars from *Fraxinus* (Fig 33). Despite not having any type material for *S. chionanthi*, we are confident in our assessment that the original species description agrees with the current understanding of *S. chionanthi* and that *S. forbesi* represents a distinct species.

S. chionanthi was described as being very rare in Georgia (Abbot and Smith 1797). There are no recent reports of *S. chionanthi* living in Georgia (J. Adams pers. comm.), and even in North Carolina it is an extremely rare mountain taxon (Bo Sullivan pers. comm.).

Although the adults of *S. forbesi* and *S. chionanthi* are difficult (and sometimes impossible) to distinguish even upon dissection, their larvae are distinct in size, coloration, habitus, and life history. Presumably these coloration and morphological differences reflect, at least in part, the structural differences in their preferred hosts. *Triosteum* is an herbaceous perennial that dies back to the ground each winter; *Fraxinus* and *Chionanthus* are trees. The brown, bark-like coloration of late instar *S. chionanthi* is suggestive that larvae rest off of foliage by day and perhaps even near the ground along the trunk or off the host in leaf litter. We know of no brown noctuoid larvae that rest

on foliage by day, and many, like *Catocala* Schrank, *Melipotis* Hübner, and *Zale* Hübner, may wander far from the foliage when not feeding. The coloration of last instar *S. forbesi* (Figs 32, 33) is reflective of its preferred resting site: the green stems of feverwort. Likewise, it is our guess that the less robust body, smaller prolegs, and reduced crochet hook number of S. *forbesi* reflect the fact that larvae rest adjacent to suitable foliage. By contrast, the caterpillars of *S. chionanthi* on a mature ash or fringetree may well have to traverse meters in search of suitable food each night. Surprisingly, no differences in mandible morphology of the two sister taxa were noted.

Despite the differences between the hosts of *S. forbesi* and *S. chionanthi*, the plants share secondary metabolites which may elucidate how the ancestral host plant switch was able to occur. Triosteum are members of the Caprifoliaceae, whereas the hosts of *S. chionanthi* (*Chionanthus* and *Fraxinus*) (Troubridge 2008, Robinson et al. 2012) are both Oleaceae. Both families are known to contain iridoid glycosides (Bowers 1991, Seigler 1998, Jensen et al. 2002, Lee et al. 2010). While larval hosts are known for only a small fraction of North American *Sympistis* (Troubridge 2008), at least among the known hosts, plants with iridoid glycosides figure prominently (Wagner et al. 2011). In western North America, *Penstemon*, in particular, well known to have iridoids (Stermitz et al. 1988, Krull and Stermitz 1998), supports numerous *Sympistis* species (DLW unpublished data).

Prior to Troubridge's (2008) oncocnemidine revision, *S. chionanthi* was classified in the monobasic genus, *Adita* Grote, 1874. Troubridge synonymized the genus into *Sympistis* and regarded *chionanthi* to be a highly derived species within *Sympistis* related to the *S. dentata* species group. In addition to the species that we describe here, there may be additional cryptic species in collections sorted as "*Adita chionanthi*." George Godfrey and Tim McCabe have beaten caterpillars of an "*Adita*" group species from *Symphoricarpos* Duhamel in the Upper Midwest. Images taken by Godfrey of these larvae closely approach those of *S. chionanthi*, but differ in having more gray in the ground color and some brick red over the dorsum. Unfortunately, neither McCabe nor Godfrey reared adults or preserved larvae. John Franclemont collected a large series of "*chionanthi*" in Montana and reared an ex ova cohort on *Fraxinus*. Based on the number of pinned specimens, larval photographs, and genitalic dissections it seems likely that Franclemont believed the Montana populations might represent a new species. Adults average larger and brighter than material from eastern North America. Three individuals of "*chionanthi*" from nearby Alberta, also included in the barcoding dataset, clustered separately from the eastern individuals. Given the above, we caution that our figured male and female genitalic preparations (Figs 15–17) for *S. chionanthi* are from central Canada; without larval or genetic data, we cannot with certainty know that these are nominate *S. chionanthi*. We were unable to find consistent differences in male or female genitalia in *S. chionanthi* (representing five states and provinces), or between *S. chionanthi* and *S. forbesi*. If our findings about the differences between *S. chionanthi* and *S. forbesi* are indicative for other members of the species group, or *Sympistis* more widely, larvae, life history data, and molecular data will be needed to tease apart the biological species in this complex.

Part of our interest in the new species derives from our desire to document instances where rates of phenotypic evolution in Lepidoptera differ markedly among life

stages. For example, in *Acronicta* Oschenehimer and some notodontid genera (e.g., *Datana* Walker and *Schizura* Doubleday) larval phenotypes differ substantially among related species that are otherwise difficult to determine using external and genitalic features of the adults. Adults of *Acronicta hastulifera* (J. E. Smith) and *A. dactylina* Grote are sometimes impossible to separate by eye or dissection, but each has a distinctive larva that readily distinguishes the second to final instars of both species (Wagner et al. 2011; Schmidt and Anweiler unpublished data). Conversely, plusiine caterpillars are remarkably undifferentiated relative to their adults, and often require microscopic examination even to make generic and tribal assignments (Crumb 1956, Lafontaine and Poole 1991). The adults of *Sympistis forbesi* and *S. chionanthi* are mixed in collections that we have examined (under the latter name). By contrast, their larvae, are immediately distinct, with the coloration of each approximating that of the stem-color of their primary hosts: feverwort (*Triosteum*) for *S. forbesi* and ash (*Chionanthus* and *Fraxinus*) for *S. chionanthi*. With careful morphological analysis, it may be possible to quantify these differing rates of phenotypic evolution within and between species using newly developed phylogenetic techniques (e.g., Adams 2013).

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References

Abbot J, Smith JE (1797) The natural history of the rarer lepidopterous insects of Georgia: including their systematic characters, the particulars of their several metamorphoses, and the plants on which they feed. Collected from the observation of Mr. John Abbot, many years resident in that country. Volume 2. London, England, 195 pp, Pl. 98.

Adams D (2013) Comparing evolutionary rates for different phenotypic traits on a phylogeny using likelihood. Systematic Biology 62: 181–192. doi: 10.1093/sysbio/sys083

Bowers MD (1991) The iridoid glycosides. In: Rosenthal G, Berenbaum M (Eds) Herbivores: Their Interaction with Plant Secondary Metabolites (2nd Ed). Academic Press Inc., San Diego, California, 297–321. doi: 10.1016/B978-0-12-597183-6.50013-9

Crumb SE (1956) The larvae of the Phalaenidae. USDA Technical Bulletin 1135. USDA, Washington, DC.

Forbes WTM (1954) The Lepidoptera of New York and Neighboring States. III. Noctuidae. Memoir 329. Cornell University Agricultural Experiment Station, Ithaca, New York.

Jensen SR, Franzyk H, Wallander E (2002) Chemotaxonomy of the Oleaceae: iridoids as taxonomic markers. Phytochemistry 60: 213–231. doi: 10.1016/S0031-9422(02)00102-4

Krull RE, Stermitz FR (1998) Trans-fused iridoid glycosides from *Penstemon mucronatus*. Phytochemistry 49: 2413–2415. doi: 10.1016/S0031-9422(98)00349-5

Lafontaine JD (2004) Noctuoidea. Noctuidae (Part), Noctuinae (Part – Noctuini). In: Hodges RW (Ed) The Moths of America North of Mexico. Fascicle. 27.1, Wedge Entomological Research Foundation, Washington, DC.

Lafontaine JD, Poole RW (1991) Noctuidae, Plusiinae. In: Dominick RB et al. (Eds) The Moths of America North of Mexico. Fascicle 25.1, Wedge Entomological Research Foundation, Washington, DC.

Lafontaine JD, Schmidt BC (2010) Annotated check list of the Noctuoidea (Insecta, Lepidoptera) of North America north of Mexico. ZooKeys 40: 1–239. doi: 10.3897/zookeys.40.414

Lee EJ, Lee JY, Kim JS, Kang SS (2010) Phytochemical studies on Lonicerae Flos–Isolation of iridoid glycosides and other constituents. Natural Products Sciences 16: 32–38.

Robinson GS, Ackery PR, Kitching IJ, Beccaloni GW, Hernández LM (2012) HOSTS - a database of the hostplants of the world's Lepidoptera. http://www.nhm.ac.uk/research-curation/projects/hostplants/

Rummel C (1921) *Adita chionanthi* A. and S. (Lepidoptera) feeding on *Triosteum perfoliatum* L. Brooklyn Entomological Society 16: 24.

Seigler DS (1998) Plant Secondary Metabolism. Kluwer Academic Publishers, Norwell, Massachusetts. doi: 10.1007/978-1-4615-4913-0

Stermitz FR, Gardner DR, McFarland N (1988) Iridoid glycoside sequestration by two aposematic *Penstemon*-feeding geometrid larvae. Journal of Chemical Ecology 14: 435–441. doi: 10.1007/BF01013895

Troubridge JT (2008) A generic realignment of the Oncocnemidini *sensu* Hodges (1983) (Lepidoptera: Noctuidae: Oncocnemidinae), with description of a new genus and 50 new species. Zootaxa 1903: 1–95.

Wagner DL (2007) Emerald ash borer threatens ash-feeding Lepidoptera. News Lepidopterists' Society 49: 10–11.

Wagner DL, Schweitzer DF, Sullivan JB, Reardon RC (2011) Owlet Caterpillars of Eastern North America. Princeton University Press, Princeton, New Jersey, 576 pp.