SI Appendix to "Genomes reveal drastic and recurrent phenotypic divergence in Firetip skipper butterflies (Hesperiidae: Pyrrhopyginae)" by Jing Zhang, Qian Cong, Jinhui Shen, Ernst Brockmann and Nick V. Grishin

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Taxonomic Appendix
T1. Taxonomic abstract

On the basis of genome-scale phylogenetic analysis, we revised higher classification of the subfamily Pyrrhopyginae Mabille, 1877 (Lepidoptera: Hesperiidae). The subfamily is partitioned into 5 tribes, one of which is new: Azonaxini Grishin, **trib. n.** and is monotypic. The largest tribe Pyrrhopygini is divided into 4 subtribes, three of which are new: Apyrrothrixina Grishin, **subtr. n.**, Mimoniadina Grishin, **subtr. n.**, and Microcerisina Grishin, **subtr. n.** Genera of Pyrrhopyginae are defined as the lineages from about 10 million years ago, which resulted in 23 genera. *Agara* Mabille & Boullet, 1908 is removed from synonymy and treated as a valid genus. The following genera are treated as subjective junior synonyms: *Cyanopyge* O. Mielke, 2002 of *Melanopyge* O. Mielke, 2002; *Mimardaris* O. Mielke, 2002 of *Ardaris* E. Watson, 1893; *Metardaris* Mabille, 1903 of *Sarbia* E. Watson, 1893; *Elbella* Evans, 1951 of *Microceris* E. Watson, 1893. In addition to genera, 22 subgenera are suggested, 10 of which are proposed as new: *Aesculapyge* Grishin, **subgen. n.** (TS: *Pyrrhopyge aesculapus* Staudinger, 1876), *Sarbiena* Grishin, **subgen. n.** (TS: *Sarbia catomelaena* Mabille & Boullet, 1908), *Santea* Grishin, **subgen. n.** (TS: *Pyrrhopyga antias* C. Felder & R. Felder, 1859), *Mimadia* Grishin, **subgen. n.** (TS: *Pyrrhopyga fallax* Mabille, 1878), *Jember* Grishin, **subgen. n.** (TS: *Jemadia scomber* Druce, 1908), *Jematus* Grishin, **subgen. n.** (TS: *Papilio gnetus* Fabricius, 1781), *Jemasonia* Grishin, **subgen. n.** (TS: *Pyrrhopyga hewitsonii* Mabille, 1878), *Merobella* Grishin, **subgen. n.** (TS: *Jemadia merops* Bell, 1934), *Blubella* Grishin, **subgen. n.** (TS: *Pyrrhopyga patroclus* Plötz, 1879), and *Apatiella* Grishin, **subgen. n.** (TS: *Hesperia iphinous* Latreille, [1924]). The following 9 subgenera have been previously treated as genera and a new status for them is suggested: *Melanopyge* O. Mielke, 2002; *Chalypyge* O. Mielke, 2002; *Sarbia* E. Watson, 1893; *Mysarbia* O. Mielke, 2002; *Amysoria* O. Mielke, 2002; *Amenis* E. Watson, 1893; *Ochropyge* O. Mielke, 2002; *Pseudocroniades* O. Mielke, 1995; *Olafia* Nemésio, 2005. Finally, *Mahotis* Watson, 1893; *Hegesippe* Evans, 1951; and *Dis* Mabille, 1889 have been removed from synonymy and treated as valid subgenera. *Pyrrhopyge guianae* E. Bell, 1932 is treated as a species and not a subspecies of *Pyrrhopyge phidias* (Linnaeus, 1758), with which it is not monophyletic. Changes (compared to the latest treatment) to result in the following 66 genus-species combinations are proposed: *Agara belti* (Godman & Salvin, 1879), *Agara perissodora* (Dyar, 1914), *Agara pegasus* (Mabille, 1903), *Agara draudti* (N. Riley, 1926), *Agara epimachia* (Herrich-Schäffer, 1869), *Agara santhilarius* (Latreille, [1824]), *Agara assaricus* (Cramer, 1779), *Agara michaeli* (Nicolay, 1975), *Agara pardalina* (C. Felder & R. Felder, 1867), *Apyrrothrix mulleri* E. Bell, 1934, *Apyrrothrix hoffmanni* (H. Freeman, 1977), *Apyrrothrix erythrosticta* (Godman & Salvin, 1879), *Apyrrothrix maculosa* (Hewitson, 1866), *Apyrrothrix cossea* (H. Druce, 1875), *Apyrrothrix sangaris* (Skinner, 1921), *Apyrrothrix chalybea* (Scudder, 1872), *Apyrrothrix hygieia* (C. Felder & R. Felder, 1867), *Apyrrothrix zereda* (Hewitson, 1866), *Apyrrothrix aesculapus* (Staudinger, 1876), *Ardaris aerata* (Godman & Salvin, 1879), *Ardaris sela* (Hewitson, 1866), *Ardaris lomax* (Evans, 1951), *Ardaris montra* (Evans, 1951), *Ardaris pityusa* (Hewitson, 1857), *Ardaris porus* (Plötz, 1879), *Ardaris minthe* (Godman & Salvin, 1879), *Mysoria cosinga* (Hewitson, 1874), *Mysoria xanthippe* (Latreille, [1824]), *Mysoria damippe* (Mabille & Boullet, 1908), *Mysoria pertyi* (Plötz, 1879), *Mysoria curitiba* (O. Mielke & Casagrande, 2002), *Mysoria soza* (Evans, 1951), *Mysoria oneka* (Hewitson, 1866), *Mysoria catomelaena* (Mabille & Boullet, 1908), *Mysoria antias* (C. Felder & R. Felder, 1859), *Mysoria sejanus* (Hopffer, 1874), *Mysoria galgala* (Hewitson, 1866), *Mimoniades pionia* (Hewitson, 1857), *Mimoniades rogeri* (Orellana, [2010]), *Mimoniades ponina* (Herrich-Schäffer, 1869), *Mimoniades fallax* (Mabille, 1878), *Protelbella ruficauda* (Hayward, 1932), *Parelbella machaon* (Westwood, 1852), *Microceris merops* (E. Bell, 1934), *Microceris patrobas* (Hewitson, 1857), *Microceris blanda* (Evans, 1951), *Microceris lustra* (Evans, 1951), *Microceris azeta* (Hewitson, 1866), *Microceris miodesmiata* (Röber, 1925), *Microceris madeira* (O. Mielke, 1995), *Microceris patroclus* (Plötz, 1879), *Microceris bicuspis* (de Jong, 1983), *Microceris rondonia* (O. Mielke, 1995), *Microceris etna* (Evans, 1951), *Microceris adonis* (E. Bell, 1931), *Microceris iphinous* (Latreille, [1824]), *Microceris mariae* (E. Bell, 1931), *Microceris luteizona* (Mabille, 1877), *Microceris hegesippe* (Mabille & Boullet, 1908), *Microceris theseus* (E. Bell, 1934), *Microceris scylla* (Ménétriés, 1855), *Microceris dulcinea* (Plötz, 1879), *Microceris intersecta* (Herrich-Schäffer, 1869), *Microceris viriditas* (Skinner, 1920), *Microceris lamprus* (Hopffer, 1874), and *Oxynetra roscius* (Hopffer, 1874). The above-listed changes are propagated to all names treated as subspecies and synonyms of these taxa, and two taxa: *Agara michaeli* (Nicolay, 1975) and *Apyrrothrix hygieia* (C. Felder & R. Felder, 1867) are treated as species, not subspecies.

T2. Treatment with intermediate genera: diverged about 10 Mya.

New tribe, subtribes and subgenera are described in the main text. Comprehensive species list can be found at <https://www.butterfliesofamerica.com/L/Hesperiidae.htm>. Below, change in taxonomic status or new taxa are indicated in red font after the name. New taxa are additionally highlighted yellow. Synonyms are denoted by "=" in front of the name (not followed by daggers are subjective junior synonyms; ‡ marks unavailable names, such as homonyms and nomina nuda) and valid names for the synonyms that are type species are shown in parenthesis.

Subfamily **Pyrrhopyginae** Mabille, 1877

Tribe **Azonaxini** Grishin, new tribe

Genus ***Azonax*** Godman & Salvin, 1893; TS: *typhaon* Hewitson, 1877; new placement, was in Passovini

Tribe **Zoniini** Mielke, 2001

Genus ***Zonia*** Evans, 1951; TS: *zonia* Evans, 1951

Tribe **Passovini** Mielke, 2001

Genus ***Granila*** Mabille, 1903; TS: *paseas* Hewitson, 1857
Genus ***Aspitha*** Evans, 1951; *aspitha* Hewitson, [1866]
Genus ***Myscelus*** Hübner, [1819]; TS: *nobilis* Cramer, [1777]
Genus ***Agara*** Mabille & Boullet, 1908; reinstated status; TS: *pardalina* C. Felder & R. Felder, 1867
Genus ***Passova*** Evans, 1951; TS: *passova* Hewitson, [1866]

Tribe **Pyrrhopygini** Mabille, 1877

Subtribe **Pyrrhopygina** Mabille, 1877
Genus ***Pyrrhopyge*** Hübner, [1819]; TS: *bixae* Linnaeus, 1758
=*Tamyris* Swainson, 1821; TS: =*zeleucus* Fabricius (*phidias* Linnaeus, 1758)
=*Pachyrhopala* Wallengren, 1858; TS: *phidias* Linnaeus, 1758
Genus ***Gunayan*** O. Mielke, 2002; TS: *rhacia* Hewitson, 1875
Genus ***Yanguna*** E. Watson, 1893; TS: *spatiosa* Hewitson, 1870

Subtribe **Apyrrothrixina** Grishin, new subtribe
Genus ***Apyrrothrix*** Lindsey, 1921; TS: *araxes* Hewitson, 1867
Subgenus *Apyrrothrix* Lindsey, 1921; TS: *araxes* Hewitson, 1867
Subgenus *Melanopyge* O. Mielke, 2002; new status; TS: *maculosa* Hewitson, 1866
=*Cyanopyge* O. Mielke, 2002; new synonym; TS: *sangaris* Skinner, 1921
Subgenus *Chalypyge* O. Mielke, 2002; new status; TS: *chalybea* Scudder, 1872
Subgenus *Aesculapyge* Grishin, new subgenus; TS: *aesculapus* Staudinger, 1876
Genus ***Creonpyge*** O. Mielke, 2002; TS: *creon* Druce, 1874
Genus ***Jonaspyge*** O. Mielke, 2002; TS: *jonas* C. & R. Felder, 1859

Subtribe **Mimoniadina** Grishin, new subtribe
Genus ***Ardaris*** E. Watson, 1893; TS: *eximia* Hewitson, 1871
=*Mimardaris* O. Mielke, 2002; new synonym; TS: *sela* Hewitson, 1866
Genus ***Mysoria*** E. Watson, 1893; TS: =‡*acastus* Cramer, [1775] (*barcastus* Sepp, [1851])
Subgenus *Sarbia* E. Watson, 1893; new status; TS: *xanthippe* Latreille, [1824]
=*Metardaris* Mabille, 1903; new synonym; TS: *cosinga* Hewitson, 1874
Subgenus *Sarbiena* Grishin, new subgenus; TS: *catomelaena* Mabille & Boullet, 1908
Subgenus *Santea* Grishin, new subgenus; TS: *antias* C. Felder & R. Felder, 1859
Subgenus *Mysarbia* O. Mielke, 2002; new status; TS: *sejanus* Hopffer, 1874
Subgenus *Mysoria* E. Watson, 1893; new status; TS: =‡*acastus* Cramer, [1775] (*barcastus* Sepp, [1851])
Subgenus *Amysoria* O. Mielke, 2002; new status; TS: *galgala* Hewitson, [1866]
Genus ***Mimoniades*** Hübner, 1823; TS: *ocyalus* Hübner, 1823
Subgenus *Amenis* E. Watson, 1893; new status; TS: *pionia* Hewitson, 1857

Subgenus *Mahotis* Watson, 1893; new status; TS: *nurscia* Swainson, 1821
Subgenus *Mimoniades* Hübner, 1823; TS: *ocyalus* Hübner, 1823
Subgenus *Mimadia* Grishin, new subgenus; TS: *fallax* Mabille, 1878
Genus ***Jemadia*** E. Watson, 1893; TS: *hospita* Butler, 1877
Subgenus *Jember* Grishin, new subgenus; TS: *scomber* H. Druce, 1908
Subgenus *Jemadia* E. Watson, 1893; TS: *hospita* Butler, 1877
Subgenus *Jematus* Grishin, new subgenus; TS: *gnetus* Fabricius, 1781
Subgenus *Jemasonia* Grishin, new subgenus; TS: *hewitsonii* Mabille, 1878
Genus ***Nosphistia*** Mabille & Boullet, 1908; TS: =*perplexus* Mabille, 1878 (*zonara* Hewitson, [1866])

Subtribe **Microcerisina** Grishin, new subtribe
Genus ***Croniades*** Mabille, 1903; TS: *pieria* Hewitson, 1857
Genus ***Protelbella*** O. Mielke, 1995; TS: *alburna* Mabille, 1891
Subgenus *Ochropyge* O. Mielke, 2002; new status; TS: *ruficauda* Hayward, 1932
Subgenus *Protelbella* O. Mielke, 1995; TS: *alburna* Mabille, 1891
Genus ***Parelbella*** O. Mielke, 1995; TS: *polyzona* Latreille, 1824
Subgenus *Pseudocroniades* O. Mielke, 1995; new status; TS: *machaon* Westwood, 1852
Subgenus *Parelbella* O. Mielke, 1995; TS: *polyzona* Latreille, 1824
Genus ***Microceris*** E. Watson, 1893; TS: *variicolor* Ménétriés, 1855
Subgenus *Merobella* Grishin, new subgenus; TS: *merops* E. Bell, 1934
Subgenus *Blubella* Grishin, new subgenus; TS: *patroclus* Plötz, 1879
Subgenus *Apatiella* Grishin, new subgenus; TS: *iphinous* Latreille, [1824]
Subgenus *Hegesippe* Evans, 1951; new status; TS: *hegesippe* Mabille & Boullet, 1908
Subgenus *Microceris* E. Watson, 1893; TS: *variicolor* Ménétriés, 1855
=*Elbella* Evans, 1951; new synonym; TS: *scylla* Ménétriés, 1855

Tribe **Oxynetrini** Mielke, 2001

Genus ***Oxynetra*** C. Felder & R. Felder, 1862; TS: *semihyalina* C. Felder & R. Felder, 1862
Subgenus *Olafia* Nemésio, 2005; new status; TS: *roscius* Hopffer, 1874
Subgenus *Dis* Mabille, 1889; new status; TS: =*annulatus* Mabille 1889 (*hopfferi* Staudinger, 1888)
Subgenus *Oxynetra* C. Felder & R. Felder, 1862; TS: *semihyalina* C. Felder & R. Felder, 1862

T3. Treatment with broader genera: diverged about 15 Mya.

Subfamily **Pyrrhopyginae** Mabille, 1877

Tribe **Azonaxini** Grishin, new tribe

Genus ***Azonax*** Godman & Salvin, 1893; TS: *typhaon* Hewitson, 1877

Tribe **Zoniini** Mielke, 2001

Genus ***Zonia*** Evans, 1951; TS: *zonia* Evans, 1951

Tribe **Passovini** Mielke, 2001

Genus ***Myscelus*** Hübner, [1819]; TS: *nobilis* Cramer, [1777]
- Subgenus *Granila* Mabille, 1903; TS: *paseas* Hewitson, 1857
- Subgenus *Aspitha* Evans, 1951; *aspitha* Hewitson, [1866]
- Subgenus *Myscelus* Hübner, [1819]; TS: *nobilis* Cramer, [1777] (related to *Granila* and *Aspitha*)
- Subgenus *Agara* Mabille & Boullet, 1908; TS: *pardalina* C. Felder & R. Felder, 1867 (related to *Passova*)
- Subgenus *Passova* Evans, 1951; TS: *passova* Hewitson, [1866]

Tribe **Pyrrhopygini** Mabille, 1877

Genus ***Pyrrhopyge*** Hübner, [1819]; TS: *bixae* Linnaeus, 1758
- Subgenus *Pyrrhopyge* Hübner, [1819]; TS: *bixae* Linnaeus, 1758
  - =*Tamyris* Swainson, 1821; TS: =*zeleucus* Fabricius (*phidias* Linnaeus, 1758)
  - =*Pachyrhopala* Wallengren, 1858; TS: *phidias* Linnaeus, 1758
- Subgenus *Gunayan* O. Mielke, 2002; TS: *rhacia* Hewitson, 1875
- Subgenus *Yanguna* E. Watson, 1893; TS: *spatiosa* Hewitson, 1870

Genus ***Apyrrothrix*** Lindsey, 1921; TS: *araxes* Hewitson, 1867
- Subgenus *Apyrrothrix* Lindsey, 1921; TS: *araxes* Hewitson, 1867
- Subgenus *Melanopyge* O. Mielke, 2002; TS: *maculosa* Hewitson, 1866
  - =*Cyanopyge* O. Mielke, 2002; TS: *sangaris* Skinner, 1921
- Subgenus *Chalypyge* O. Mielke, 2002; TS: *chalybea* Scudder, 1872
- Subgenus *Aesculapyge* Grishin, new subgenus; TS: *aesculapus* Staudinger, 1876
- Subgenus *Creonpyge* O. Mielke, 2002; TS: *creon* Druce, 1874
- Subgenus *Jonaspyge* O. Mielke, 2002; TS: *jonas* C. & R. Felder, 1859

Genus ***Mimoniades*** Hübner, 1823; TS: *ocyalus* Hübner, 1823
- Subgenus *Ardaris* E. Watson, 1893; TS: *eximia* Hewitson, 1871
  - =*Mimardaris* O. Mielke, 2002; TS: *sela* Hewitson, 1866
- Subgenus *Sarbia* E. Watson, 1893; TS: *xanthippe* Latreille, [1824]
  - =*Metardaris* Mabille, 1903; TS: *cosinga* Hewitson, 1874
- Subgenus *Sarbiena* Grishin, new subgenus; TS: *catomelaena* Mabille & Boullet, 1908
- Subgenus *Santea* Grishin, new subgenus; TS: *antias* C. Felder & R. Felder, 1859
- Subgenus *Mysarbia* O. Mielke, 2002; TS: *sejanus* Hopffer, 1874
- Subgenus *Mysoria* E. Watson, 1893; TS: =‡*acastus* Cramer, [1775] (*barcastus* Sepp, [1851])
- Subgenus *Amysoria* O. Mielke, 2002; TS: *galgala* Hewitson, [1866]
- Subgenus *Amenis* E. Watson, 1893; TS: *pionia* Hewitson, 1857
- Subgenus *Mahotis* Watson, 1893; TS: *nurscia* Swainson, 1821
- Subgenus *Mimoniades* Hübner, 1823; TS: *ocyalus* Hübner, 1823
- Subgenus *Mimadia* Grishin, new subgenus; TS: *fallax* Mabille, 1878
- Subgenus *Jember* Grishin, new subgenus; TS: *scomber* H. Druce, 1908
- Subgenus *Jemadia* E. Watson, 1893; TS: *hospita* Butler, 1877
- Subgenus *Jematus* Grishin, new subgenus; TS: *gnetus* Fabricius, 1781
- Subgenus *Jemasonia* Grishin, new subgenus; TS: *hewitsonii* Mabille, 1878
- Subgenus *Nosphistia* Mabille & Boullet, 1908; TS: =*perplexus* Mabille, 1878 (*zonara* Hewitson, [1866])

Genus ***Microceris*** E. Watson, 1893; TS: *variicolor* Ménétriés, 1855
- Subgenus *Croniades* Mabille, 1903; TS: *pieria* Hewitson, 1857

Subgenus *Ochropyge* O. Mielke, 2002; TS: *ruficauda* Hayward, 1932
Subgenus *Protelbella* O. Mielke, 1995; TS: *alburna* Mabille, 1891
Subgenus *Pseudocroniades* O. Mielke, 1995; TS: *machaon* Westwood, 1852
Subgenus *Parelbella* O. Mielke, 1995; TS: *polyzona* Latreille, 1824
Subgenus *Merobella* Grishin, new subgenus; TS: *merops* E. Bell, 1934
Subgenus *Blubella* Grishin, new subgenus; TS: *patroclus* Plötz, 1879
Subgenus *Apatiella* Grishin, new subgenus; TS: *iphinous* Latreille, [1824]
Subgenus *Hegesippe* Evans, 1951; TS: *hegesippe* Mabille & Boullet, 1908
Subgenus *Microceris* E. Watson, 1893; TS: *variicolor* Ménétriés, 1855
=*Elbella* Evans, 1951; TS: *scylla* Ménétriés, 1855

Tribe **Oxynetrini** Mielke, 2001

Genus ***Oxynetra*** C. Felder & R. Felder, 1862; TS: *semihyalina* C. Felder & R. Felder, 1862
Subgenus *Olafia* Nemésio, 2005; TS: *roscius* Hopffer, 1874
Subgenus *Dis* Mabille, 1889; TS: =*annulatus* Mabille 1889 (*hopfferi* Staudinger, 1888)
Subgenus *Oxynetra* C. Felder & R. Felder, 1862; TS: *semihyalina* C. Felder & R. Felder, 1862

T4. Treatment with narrower genera: diverged about 5 Mya.

Subfamily **Pyrrhopyginae** Mabille, 1877

Tribe **Azonaxini** Grishin, new tribe

Genus ***Azonax*** Godman & Salvin, 1893; TS: *typhaon* Hewitson, 1877

Tribe **Zoniini** Mielke, 2001

Genus ***Zonia*** Evans, 1951; TS: *zonia* Evans, 1951

Tribe **Passovini** Mielke, 2001

Genus ***Granila*** Mabille, 1903; TS: *paseas* Hewitson, 1857
Genus ***Aspitha*** Evans, 1951; *aspitha* Hewitson, [1866]
Genus ***Myscelus*** Hübner, [1819]; TS: *nobilis* Cramer, [1777]
Genus ***Agara*** Mabille & Boullet, 1908; TS: *pardalina* C. Felder & R. Felder, 1867
Genus ***Passova*** Evans, 1951; TS: *passova* Hewitson, [1866]

Tribe **Pyrrhopygini** Mabille, 1877

Subtribe **Pyrrhopygina** Mabille, 1877

Genus ***Pyrrhopyge*** Hübner, [1819]; TS: *bixae* Linnaeus, 1758
=*Tamyris* Swainson, 1821; TS: =*zeleucus* Fabricius (*phidias* Linnaeus, 1758)
=*Pachyrhopala* Wallengren, 1858; TS: *phidias* Linnaeus, 1758
Genus ***Gunayan*** O. Mielke, 2002; TS: *rhacia* Hewitson, 1875
Genus ***Yanguna*** E. Watson, 1893; TS: *spatiosa* Hewitson, 1870

Subtribe **Apyrrothrixina** Grishin, new subtribe

Genus ***Apyrrothrix*** Lindsey, 1921; TS: *araxes* Hewitson, 1867
Genus ***Melanopyge*** O. Mielke, 2002; TS: *maculosa* Hewitson, 1866
=*Cyanopyge* O. Mielke, 2002; TS: *sangaris* Skinner, 1921
Genus ***Chalypyge*** O. Mielke, 2002; TS: *chalybea* Scudder, 1872
Genus ***Aesculapyge*** Grishin; TS: *aesculapus* Staudinger, 1876
Genus ***Creonpyge*** O. Mielke, 2002; TS: *creon* Druce, 1874
Genus ***Jonaspyge*** O. Mielke, 2002; TS: *jonas* C. & R. Felder, 1859

Subtribe **Mimoniadina** Grishin, new subtribe

Genus ***Ardaris*** E. Watson, 1893; TS: *eximia* Hewitson, 1871
=*Mimardaris* O. Mielke, 2002; TS: *sela* Hewitson, 1866
Genus ***Sarbia*** E. Watson, 1893; TS: *xanthippe* Latreille, [1824]
=*Metardaris* Mabille, 1903; TS: *cosinga* Hewitson, 1874
Genus ***Sarbiena*** Grishin; TS: *catomelaena* Mabille & Boullet, 1908
Genus ***Santea*** Grishin; TS: *antias* C. Felder & R. Felder, 1859
Genus ***Mysarbia*** O. Mielke, 2002; TS: *sejanus* Hopffer, 1874
Genus ***Mysoria*** E. Watson, 1893; TS: =‡*acastus* Cramer, [1775] (*barcastus* Sepp, [1851])
Genus ***Amysoria*** O. Mielke, 2002; TS: *galgala* Hewitson, [1866]
Genus ***Amenis*** E. Watson, 1893; TS: *pionia* Hewitson, 1857
Genus ***Mahotis*** Watson, 1893; TS: *nurscia* Swainson, 1821
Genus ***Mimoniades*** Hübner, 1823; TS: *ocyalus* Hübner, 1823
Genus ***Jember*** Grishin; TS: *scomber* H. Druce, 1908
Genus ***Mimadia*** Grishin; TS: *fallax* Mabille, 1878
Genus ***Jemadia*** E. Watson, 1893; TS: *hospita* Butler, 1877
Genus ***Jematus*** Grishin; TS: *gnetus* Fabricius, 1781
Genus ***Jemasonia*** Grishin; TS: *hewitsonii* Mabille, 1878
Genus ***Nosphistia*** Mabille & Boullet, 1908; TS: =*perplexus* Mabille, 1878 (*zonara* Hewitson, [1866])

Subtribe **Microcerisina** Grishin, new subtribe

Genus ***Croniades*** Mabille, 1903; TS: *pieria* Hewitson, 1857
Genus ***Ochropyge*** O. Mielke, 2002; TS: *ruficauda* Hayward, 1932
Genus ***Protelbella*** O. Mielke, 1995; TS: *alburna* Mabille, 1891
Genus ***Pseudocroniades*** O. Mielke, 1995; TS: *machaon* Westwood, 1852
Genus ***Parelbella*** O. Mielke, 1995; TS: *polyzona* Latreille, 1824
Genus ***Merobella*** Grishin; TS: *merops* E. Bell, 1934
Genus ***Blubella*** Grishin; TS: *patroclus* Plötz, 1879
Genus ***Apatiella*** Grishin; TS: *iphinous* Latreille, [1824]
Genus ***Hegesippe*** Evans, 1951; TS: *hegesippe* Mabille & Boullet, 1908
Genus ***Microceris*** E. Watson, 1893; TS: *variicolor* Ménétriés, 1855
=*Elbella* Evans, 1951; TS: *scylla* Ménétriés, 1855

Tribe **Oxynetrini** Mielke, 2001

Genus ***Olafia*** Nemésio, 2005; TS: *roscius* Hopffer, 1874
Genus ***Dis*** Mabille, 1889; TS: =*annulatus* Mabille 1889 (*hopfferi* Staudinger, 1888)
Genus ***Oxynetra*** C. Felder & R. Felder, 1862; TS: *semihyalina* C. Felder & R. Felder, 1862

T5. Expanded morphological diagnoses for the new taxa described in the main text

Page limits on the main text forced us to keep diagnoses minimal. In the main text, the words for diagnostic DNA characters were given as abbreviations. While such descriptions were sufficient to define these taxa, morphological characters are desirable in addition to DNA. To provide morphological definition of each taxon within minimal space, the Evans (1951) key was referenced. When space allowed, the most prominent synapomorphic character of genitalia was specified. Here, morphological characters are spelled out in more detail, as well as some other additional information.

Tribe Azonaxini Grishin, trib. n.

Type genus: *Azonax* Godman & Salvin, 1893. **ZooBank registration:** 6E3B9F8E-91C0-45BD-AF1F-78C5F2769392

Diagnosis: Differs from other Pyrrhopyginae by a combination of divided, U-shaped uncus with I-shaped gnathos (a possible synapomorphy), and with antennal club bent to form apiculus at its thickest part, not before it. Valva longer than wide, harpe shorter than half of valva length, with dorsal tooth. In contrast, while uncus divided in its sister tribe Zoniini, gnathos U-shaped, and Passovini possess undivided uncus and lack gnathos. Forewings with apex more pointed than in all other Pyrrhopyginae but Zoniini, from which it differs by truncate and excavate forewing apex (=falcate) and spotted, not striped, wing pattern.

Genera included: *Azonax* Godman & Salvin, 1893.

Parent Taxon: Subfamily Pyrrhopyginae Mabille, 1877.

Subtribe Apyrrothrixina Grishin, subtr. n.

Type genus: *Apyrrothrix* Lindsey, 1921. **ZooBank registration:** 8EEE17EE-CCD5-4A4A-9105-0F81E498C6FD

Diagnosis: Differs from its relatives by the following combination of characters. Antennal club bent to form apiculus before its thickest part, apiculus gradually tapering to sharp point, discocellular vein on hindwing concave towards outer margin, veins CuA_1 & M_3 and M_1 & RS wide apart at their origins, end of abdomen brown (if red-orange, then wings unspotted and hindwing crenulate at outer margin and fringes frequently white), sides of abdomen without red stripes at segments (if orange-striped, then stripes extend on abdomen below and bases of both wings orange below), if head with white or yellow lines and dots, then hindwing crenulate, if head unspotted, then fringes not white.

Genera included: *Apyrrothrix* Lindsey, 1921 (with subgenera: *Melanopyge* O. Mielke, 2002 [with junior subjective synonym *Cyanopyge* O. Mielke, 2002], *Chalypyge* O. Mielke, 2002, and *Aesculapyge* Grishin, subgen. n.), *Creonpyge* O. Mielke, 2002, and *Jonaspyge* O. Mielke, 2002.

Parent Taxon: Tribe Pyrrhopygini Mabille, 1877.

Subtribe Mimoniadina Grishin, subtr. n.

Type genus: *Mimoniades* Hübner, 1823. **ZooBank registration:** D71E4FF9-F89D-4BE1-ABB1-F86F08B98817

Diagnosis: Differs from its relatives by the following combination of characters. Antennal club bent to form apiculus before its thickest part, in most species apiculus tapering only near its blunt or rounded tip, hindwing margin not crenulate. If apiculus gradually tapering to a point, then hindwing veins CuA_1 & M_3 close to each other at their origins and forewing vein M_3 in the middle between veins M_2 and CuA_1 at their origins (not between veins M_1 and CuA_1).

Genera included: *Ardaris* E. Watson, 1893 (with junior subjective synonym *Mimardaris* O. Mielke, 2002), *Mysoria* E. Watson, 1893 (with subgenera: *Sarbia* E. Watson, 1893 [with junior subjective synonym *Metardaris* Mabille, 1903], *Sarbiena* Grishin, subgen. n., *Santea* Grishin, subgen. n., *Mysarbia* O. Mielke, 2002, and *Amysoria* O. Mielke, 2002), *Mimoniades* Hübner, 1823 (with subgenera: *Amenis* E. Watson, 1893, *Mahotis*

Watson, 1893, and *Mimadia* Grishin, subgen. n.), *Jemadia* E. Watson, 1893 (with subgenera: *Jember* Grishin, subgen. n., *Jematus* Grishin, subgen. n., and *Jemasonia* Grishin, subgen. n.), and *Nosphistia* Mabille & Boullet, 1908.

Parent Taxon: Tribe Pyrrhopygini Mabille, 1877.

Subtribe Microcerisina Grishin, subtr. n.

Type genus: *Microceris* E. Watson, 1893. **ZooBank registration:** 2D1DB769-9A47-4BD1-A66D-9CD937825113

Diagnosis: Defined as "Elbella complex" of Mielke (1995) after addition of *Ochropyge* (and placing it as a subgenus of *Protelbella*) and distinguished from its relatives by a likely synapomorphy: lateral lobe at distal end of aedeagus, apparently to support vesica.

Genera included: *Croniades* Mabille, 1903, *Protelbella* O. Mielke, 1995 (with subgenus *Ochropyge* O. Mielke, 2002), *Parelbella* O. Mielke, 1995 (with subgenus *Pseudocroniades* O. Mielke, 1995), and *Microceris* E. Watson, 1893 (with junior subjective synonym *Elbella* Evans, 1951 and subgenera: *Merobella* Grishin, subgen. n., *Blubella* Grishin, subgen. n., *Apatiella* Grishin, subgen. n., and *Hegesippe* Evans, 1951).

Parent Taxon: Tribe Pyrrhopygini Mabille, 1877.

Subgenus *Aesculapyge* Grishin, subgen. n.

Type species: *Pyrrhopyge aesculapus* Staudinger, 1876. **ZooBank regist.:** D6952953-3744-402D-9A01-9D88246DAB47

Diagnosis: Distinguished from its relatives by shiny metallic-blue wings with somewhat crenulate hindwing margins, no orange on body, and orange hindwing fringes, black on forewing. Harpe elongated, narrower than in relatives, smoothly curved dorsad, C-shaped, rounded at the tip, with a tooth at it base.

Species included: *Pyrrhopyge aesculapus* Staudinger, 1876.

Parent Taxon: Genus *Apyrrothrix* Lindsey, 1921.

Subgenus *Sarbiena* Grishin, subgen. n.

Type species: *Sarbia catomelaena* Mabille & Boullet, 1908. **ZooBank reg.:** D76F2A12-DB82-46E3-A06E-3EA038A0B0E0

Diagnosis: Distinguished from its relatives by hind tibiae lacking upper pair of spurs, black tegulae, narrow yellow bands with irregular margins particularly on hindwing, hindwing below with a basal yellow spot in cell C-Sc+R_1, palpi terminally orange-red. Uncus broad, lacks dorsally directed spike, arms long, distant from each other.

Species included: *Sarbia catomelaena* Mabille & Boullet, 1908.

Parent Taxon: Genus *Mysoria* E. Watson, 1893.

Subgenus *Santea* Grishin, subgen. n.

Type species: *Pyrrhopyga* [sic] *antias* C. & R. Felder, 1859. **ZooBank regist.:** 86F43126-2F5D-491C-A6A5-C0CCC584E278

Diagnosis: Distinguished from its relatives by hind tibiae lacking upper pair of spurs, black tegulae, narrow yellow bands with very regular margins particularly on hindwing, hindwing below without basal yellow spot, palpi terminally black. Uncus narrow, with a spike directed dorsad, arms short, near each other.

Species included: *Pyrrhopyga* [sic] *antias* C. & R. Felder, 1859.

Parent Taxon: Genus *Mysoria* E. Watson, 1893.

Subgenus *Mimadia* Grishin, subgen. n.

Type species: *Pyrrhopyga*[sic] *fallax* Mabille, 1878. **ZooBank registration**: D9680514-89C4-42B8-BA11-F36A628652C8

Diagnosis: Distinguished from its relatives by long central blue band on hindwing from vein Rs to 1A+2A, well-developed submarginal blue band, lacking basal white streaks (just white area), submarginal forewing blue band touching hyaline spots in cells M_3-CuA_1 and M_1-M_2, and white-lined patagia. Genitalic valvae asymmetrical, both harpes rounded, right harpe with more concave dorsal margin than left harpe. Formerly and incorrectly placed in *Jemadia* due to similarities in wing patterns: *Jemadia* species possess symmetrical genitalia.

Species included: *Pyrrhopyga* [sic] *fallax* Mabille, 1878.

Parent Taxon: Genus *Mimoniades* Hübner, 1823.

Subgenus *Jematus* Grishin, subgen. n.

Type species: *Papilio gnetus* Fabricius, 1781. **ZooBank registration**: 8CE439F0-2FC8-4D67-BA9A-CB90CB47B447

Diagnosis: Distinguished from its relatives by long central blue band on hindwing from vein Rs to 1A+2A, well-developed submarginal blue band, lacking basal white streaks (just white area), submarginal forewing blue band passing distad of hyaline spots in cells M_3-CuA_1 and M_1-M_2, not touching them, and white-lined patagia. Genitalic harpe triangular, with basal process and small tooth separated from it by narrow indentation, ampulla straight, no tooth.

Species included: *Papilio gnetus* Fabricius, 1781 and *Jemadia brevipennis* Schaus, 1902.

Parent Taxon: Genus *Jemadia* E. Watson, 1893.

Subgenus *Jember* Grishin, subgen. n.

Type species: *Jemadia scomber* Druce, 1908. **ZooBank registration**: BD5E8AE8-1F65-4580-8288-2507928612D4

Diagnosis: Distinguished from its relatives by the absence of central blue band on hindwing above, hindwing only with submarginal blue band (sometimes close to wing center) and basal streaks and white areas, submarginal forewing blue band passing distad of hyaline spots in cells M_3-CuA_1 and M_1-M_2, not touching them. Genitalic harpe bent dorsad, not tapering, no tooth at its base, ampulla with a tooth.

Species included: *Jemadia scomber* Druce, 1908 and *Pyrrhopyga* [sic] *menechmus* Mabille, 1878.

Parent Taxon: Genus *Jemadia* E. Watson, 1893.

Subgenus *Jemasonia* Grishin, subgen. n.

Type species: *Pyrrhopyga* [sic] *hewitsonii* Mabille, 1878. **ZooBank regist.**: 06E23C76-CCCF-4DBF-9129-6207C8315FCD

Diagnosis: Distinguished from its relatives by a short discal blue band on hindwing above, from vein Rs to vein CuA_1, caudad of two whitish basal streaks (giving appearance of 3 rays on hindwing), submarginal forewing blue band touching hyaline spots in cells M_3-CuA_1 and M_1-M_2, and white-spotted patagia. Genitalic harpe terminally upturned, nearly trapezoidal, with serrated dorsal margin, ampulla rounded, no tooth.

Species included: *Pyrrhopyga* [sic] *hewitsonii* Mabille, 1878, *Jemadia hewitsonii ovid* Evans, 1951, *Jemadia suekentonmiller* Grishin, 2014, *Jemadia hewitsonii pater* Evans, 1951, *Jemadia ortizi* Orellana, [2010], and *Jemadia albescens* Röber, 1925.

Parent Taxon: Genus *Jemadia* E. Watson, 1893.

Subgenus *Merobella* Grishin, subgen. n.

Type species: *Jemadia merops* E. Bell, 1934. **ZooBank registration**: 227201CF-9B7E-4413-9624-A976A5045620

Diagnosis: Characterized by a terminally bulbous, spoon-shaped harpe and elongated processes of tegumen, blue-striped and white-spotted wings.

Species included: *Jemadia merops* E. Bell, 1934.

Parent Taxon: Genus *Microceris* E. Watson, 1893.

Subgenus *Blubella* Grishin, subgen. n.

Type species: *Pyrrhopyga* [sic] *patroclus* Plötz, 1879. **ZooBank registration**: 373B8338-3A00-418E-A196-FF6312C88C2C

Diagnosis: Distinguished from its relatives by elongated and tapered genitalic harpe (in some species ventrally indented, C-shaped, but not thin and curved ventrad) with small projections at its base near ampulla, most species with blue-striped and white-spotted wings.

Species included: *Pyrrhopyga* [sic] *patroclus* Plötz, 1879, *Pyrrhopyga* [sic] *patrobas* Hewitson, 1857, *Elbella patrobas blanda* Evans, 1951, *Elbella azeta lustra* Evans, 1951, *Pyrrhopyga* [sic] *azeta* Hewitson, 1866, *Jemadia miodesmiata* Röber, 1925, *Elbella madeira* Mielke, 1995, *Elbella bicuspis* de Jong, 1983, *Elbella rondonia* Mielke, 1995, *Elbella etna* Evans, 1951, and *Pyrrhopyge adonis* Bell, 1931.

Parent Taxon: Genus *Microceris* E. Watson, 1893.

Subgenus *Apatiella* Grishin, subgen. n.

Type species: *Hesperia iphinous* Latreille, [1924]. **ZooBank registration**: 62010BD5-793C-429F-90DB-AA702B3C91EB

Diagnosis: Distinguished from its relatives by a terminally forked genitalic harpe, nearly T-shaped, and short and rounded processes of tegumen. Harpe somewhat similar in *Parelbella*, but more robust, and tegumen processes elongated or absent. Wings black or yellow-spotted.

Species included: *Hesperia iphinous* Latreille, [1924] and *Pyrrhopyge mariae* Bell, 1931.

Parent Taxon: Genus *Microceris* E. Watson, 1893.

References:

Evans WH. 1951. A catalogue of the American Hesperiidae indicating the classification and nomenclature adopted in the British Museum (Natural History). Part I. Introduction and Group A Pyrrhopyginae. London, British Museum (Natural History). x + 92 pp., pls. 1-9.

Mielke OHH. 1995. Revisão de *Elbella* Evans e gêneros afins (Lepidoptera, Hesperiidae, Pyrrhopyginae). *Revista brasileira de Zoologia* 11(3), 395-586.

T6. Dated genomic tree of Pyrrhopyginae and tribes

A genomic tree constructed on the concatenated alignment of protein-coding genes was dated and is shown in Fig. 1 (main text). Most internal nodes received 100% bootstrap support and represent highly reliable groups. In a few instances the order of branching is not confident due to short internal nodes. E.g., while *Pyrrhopyge*, *Gunayan* and *Yanguna* form a strongly supported monophyletic group, it is unclear whether *Yanguna*, *Gunayan* or *Pyrrhopyge* is the sister to the remaining two taxa of this group ('bootstrap' 0.48).

Major branches near the base of the tree (main text Fig. 1) correspond to Passovini, Pyrrhopygini and Oxynetrini. Interestingly, *Azonax* is not placed in Passovini, but instead is confidently grouped with *Zonia*. Both of these genera diverged soon after their divergence from Passovini. Therefore, we agree that *Zonia* is best classified in a monotypic tribe Zoniini. *Azonax* is equidistant from other taxa, and a new monotypic tribe Azonaxini is proposed for it here (main text). While there is some superficial wing pattern and color resemblance between *Azonax* and *Myscelus* as suggested previously (Evans 1951), a more careful inspection of morphology agrees with the genomic analysis. E.g., uncus in male genitalia is undivided in Passovini (including *Myscelus*), but is divided in both *Azonax* and *Zonia*. Forewing is similarly pointed at the apex in both *Azonax* and *Zonia* but is more rounded in Passovini. Gnatos is U-shaped in *Zonia*, but is I-shaped in *Azonax* and is absent in Passovini.

Oxynetrini is sister to Pyrrhopygini as suggested by Mielke's morphological analysis (Mielke 2001), but Zoniini + Azonaxini clade is sister to Passovini rather than to Oxynetrini + Pyrrhopygini. This first bifurcation of Pyrrhopyginae into the clades Zoniini + Azonaxini + Passovini and Oxynetrini + Pyrrhopygini makes morphological sense. The former clade is characterized by typically narrower genitalic valva with smaller and unmodified, simpler harpe. The harpe is expanded and frequently armed with projections in the latter clade.

T7. Pyrrhopyginae genera and inconsistencies with the current classification

Overall, we observe excellent agreement between our phylogenetic tree (main text Fig. 1) and the current classification of Pyrrhopyginae (Mielke 2005), thus largely confirming it. However, we found several polyphyletic and paraphyletic genera that we refine to ensure monophyly of all Pyrrhopyginae genera. These results were consistent in all different trees we have obtained (Figs. S2-S7).

Myscelus is polyphyletic. Strongly supported by 100% bootstrap, some species currently placed in *Myscelus* are grouped with *Aspitha* + *Granila*, while others are closely grouped with *Passova*. To restore monophyly, we resurrect *Agara* from synonymy, and transfer relatives of *Passova* into that genus, accordingly with its type species.

Jonaspyge is polyphyletic. Although only three species were placed in *Jonaspyge*, two of which are very closely related sisters, the third species, *Jonaspyge aesculapus*, does not group with them and is a sister to four other genera that form a monophyletic group (*Apyrrothrix*, *Melanopyge*, *Cyanopyge* and *Chalypyge*). These genera and "*Jonaspyge*" *aesculapus* diverged about 10 Mya and are close relatives. Therefore, we consider them as subgenera of *Apyrrothrix*, and a new subgenus *Aesculapyge* is named here for *aesculapus* (main text). Interestingly, despite the marked differences in color patterns, *Cyanopyge* closely clusters with *Melanopyge* and they diverged about the same time as *Chalypyge chalybea* has split from *Chalypyge hygieia*.

Sarbia is polyphyletic. We find that an unusually patterned *Metardaris cosinga* is a close relative of *Sarbia xanthippe*, which, in turn, does not group with *Sarbia catomelaena*. Another monotypic genus *Mysarbia* is clustered closely with the species of the former two genera. To resolve the polyphyly it is best to consider species assigned to these three genera congeneric rather than to split *Sarbia*.

Jemadia is polyphyletic. *Jemadia* species are unified by a prominent sinimustvalge blue-black-white wing pattern shared by the large mimicry complex that includes *Phocides*, a genus from a different subfamily of skippers. This superficial similarity masks genetic divergence. Genitalia of these species differ and define 5 species groups. Four of them do not have names and are described here as subgenera (main text). One of the

groups, *Jemadia fallax*, the only *Jemadia* with asymmetric valvae, is not monophyletic with others. It confidently groups with *Mimoniades* and *Amenis*, also characterized by asymmetric valvae. Therefore, *J. fallax* does not belong to *Jemadia* and is transferred to *Mimoniades*.

Mimoniades is paraphyletic. Interestingly, *Mimoniades ocyalus*, which is the type species of *Mimoniades*, is a confident (100% bootstrap) sister to a group that in addition to other *Mimoniades* species includes *Amenis*. Thus, either *Amenis* should be considered a part of *Mimoniades*, or *Mimoniades* becomes monotypic, with other species in this genus falling in the genus *Mahotis* (type species *Mahotis nurscia*). Notably, *M. ocyalus* (main text Fig. 1, image 28) is quite similar in wing patterns to *Elbella iphinous* (main text Fig. 2k), a species to which it is not closely related.

Elbella is paraphyletic. Unexpectedly, *Microceris variicolor*, a uniquely patterned skipper currently placed in its own monotypic genus, branches deeply inside *Elbella* species and is closely related to the type species of *Elbella*, *E. scylla*. This result is very confident and even COI barcodes group *M. variicolor* with *E. scylla* and its closest allies: a 3.8% barcode difference, while different genera usually show more than 6% barcode difference. As a result, *Elbella* becomes a synonym of *Microceris*. However, we see meaningful subdivisions within the new *Microceris* (former *Elbella* + *Microceris*) that are consistent with morphological similarities, and three new subgenera are named (main text).

Ardaris and *Mimardaris* are closely related. In our trees, *Mimardaris* is paraphyletic with respect to *Mimardaris aerata*, a uniquely patterned, shiny metallic skipper without typical *Mimardaris* stripes but with genitalia similar to *Mimardaris*. However, statistical support for the paraphyly is not strong (65% bootstrap in mitogenomic tree). Nevertheless, *Mimardaris* and *Ardaris* are close relatives and inclusion of *Mimardaris* species in *Ardaris* renders the genus monophyletic.

Monotypic genera and their uniqueness. Out of 35 currently recognized Pyrrhopyginae genera, 16 (45%) are monotypic. To understand the relationships of monotypic genera with others, each one was individually analyzed. We find that 2 genera (*Zonia* and *Azonax*) do not have any close relatives and diverged more than 25 Mya. These two genera are truly unique and are placed in two monotypic tribes. Thus, they cannot be combined with any other genera. However, COI barcode analysis suggests that subspecies of *Zonia* are well-differentiated and are more likely to be full species. Thus, *Zonia* may no longer be monotypic.

Conversely, monotypic *Cyanopyge*, *Ochropyge* and *Pseudocroniades* are close sisters of *Melanopyge*, *Protelbella* (monotypic) and *Parelbella* respectively, and are better placed in synonymy. *Sarbia* is polyphyletic and combining it with closely related monotypic *Metardaris* and *Mysarbia* corrects the problem. *Elbella* is paraphyletic with respect to its close relative monotypic *Microceris*, and the two should be combined. The fate of the remaining 7 monotypic genera depends on criteria to define a genus. Taking *Elbella*, *Jemadia* (excluding *J. fallax*) and *Pyrrhopyge* (sensu stricto) as a standard for divergence within a genus, we cut the genomic tree around 10 Mya to define the genera. As a result, *Granila*, *Crenopyge*, and *Nosphistia* are kept as monotypic, but *Mysarbia*, *Amysoria*, *Olafia* and *Apyrrothrix* are combined with other genera (see SI Appendix). The cut through the tree suggests that *Amysoria*, *Mysarbia*, *Metardaris* and *Sarbia* should be placed in *Mysoria*, and *Amenis* in *Mimoniades*.

References:

Evans WH. 1951. A catalogue of the American Hesperiidae indicating the classification and nomenclature adopted in the British Museum (Natural History). Part I. Introduction and Group A Pyrrhopyginae. London, British Museum (Natural History). x + 92 pp., pls. 1-9.

Mielke OHH. 2001 Estudo cladístico e descrições de tribos em Pyrrhopyginae (Lepidoptera, Hesperiidae). *Revista brasileira de Zoologia* 18(3), 897-905.

Mielke OHH. 2005 *Catalogue of the American Hesperioidea: Hesperiidae (Lepidoptera).* Curitiba, Paraná, Brazil, Sociedade Brasileira de Zoologia; xiii + 1536 pp.

T8. Justification for changes to Pyrrhopyginae genera and species

At least one representative of every Pyrrhopyginae genus was sampled for DNA and whole genomic shotgun reads were obtained. The representative was either the type species of the genus, or its close relative as suggested by COI DNA barcodes and morphology. Genera were delineated by the cut through the timed genome-scale phylogenetic tree at about 10 Mya (Fig. 1 in the main text). Every branch crossed by the cut was defined as a genus, and the oldest name available for its species was applied to it. Composition of each genus was determined by monophyly in the nuclear and mitochondrial genome trees (Figs. 1, S2). As a result, a number of species were placed in a genus different from where they were classified prior to this study (see Figs. 1, S2, "Description of new taxa" section in the main text and Taxonomic Appendix). The most notable are the changes of status from genus to subgenus for a number of previously used genera, due to their more recent origin. Subgenera were defined in some genera as lineages from approximately 5 million years ago, but some younger lineages that were given genus status prior to this study due their distinct appearance (e.g. *Sarbia* and *Amenis*) are treated as subgenera rather than synonyms. Those 5 Mya lineages that did not have a name where named as new subgenera.

The following taxa are treated as species instead of subspecies.

Agara michaeli (Nicolay, 1975); new status, new combination
Proposed as a subspecies of *Agara* [then *Myscelus*] *assaricus* (Cramer, 1779) by Nicolay (1975: 185), who described wing pattern differences from *assaricus*. COI barcodes of *assaricus* and *michaeli* differ by 1.5% (10 differences). Type series in AMNH and USNM inspected and holotype photographed by NVG. Genome of *michaeli* holotype is sequenced in this work. Placed in the genus *Agara* Mabille & Boullet, 1908, with which *Myscelus* Hübner, [1819] is not monophyletic (Figs. 1, S2).

Pyrrhopyge guianae E. Bell, 1932; reinstated status
Treated as a subspecies of *Pyrrhopyge phidias* (Linnaeus, 1758) by Evans (1953: 233, 1951: 10) who noted genitalic differences in A.1.2(i, corrected per 1953: 233). COI barcodes of *phidias* and *guianae* holotype differ by 4.4% (29 differences) and *guianae* is not monophyletic with *phidias* in the genomic tree (Fig. 1). Holotype in AMNH photographed by NVG. Genome of *guianae* holotype is sequenced in this work.

Apyrrothrix hygieia (C. Felder & R. Felder, 1867); reinstated status, new combination
Treated as a subspecies of *Apyrrothrix* [then *Pyrrhopyge*] *hygieia* (C. Felder & R. Felder, 1867) by Evans (1951: 32, 33), who made a mistake in the date of publication listed as 1866 instead of 1867 (should be a subspecies of *Apyrrothrix zereda* (Hewitson, 1866)). In A.1.47, Evans stated consistent body color, wing pattern and genitalia differences between these taxa, including the color of coxae, palpi and collar that typically correspond to differences between species. COI barcodes of *zereda* and *hygieia* differ by 5.8% (38 differences). Types of these taxa in BMNH inspected and photographed by NVG. Placed in the genus *Apyrrothrix* Lindsey, 1921, of which *Chalypyge* O. Mielke, 2002 is a subgenus (Figs. 1, S2).

Abbreviations:
AMNH: American Museum of Natural History, New York, New York, United States
BMNH: The Natural History Museum [formerly British Museum (Natural History)], London, United Kingdom

References:
Evans WH. 1951. A catalogue of the American Hesperiidae indicating the classification and nomenclature adopted in the British Museum (Natural History). Part I. Introduction and Group A Pyrrhopyginae. London, British Museum (Natural History). x + 92 pp., pls. 1-9.

Evans WH. 1953. A catalogue of the American Hesperiidae indicating the classification and nomenclature adopted in the British Museum (Natural History). Part III (Groups E, F, G) Pyrginae. Section 2. London, British Museum (Natural History). v + 246 pp., pls. 26-53.

Nicolay SS. [1975]. Illustrations and descriptions of some Pyrrhopyginae from Panama (Hesperiidae). Journal of Research on the Lepidoptera 13(3): 181-190, 9 figs.

T9. Taxonomic discussion

What is a genus?

As with tribes and subfamilies, what constitutes a genus remains undefined. Talavera et al. (2012) suggested that a cut of phylogenetic tree at a certain level that maximizes agreement with the currently accepted genera in a group of organisms may offer some objectivity. Such a cut will make genera consistent across the higher taxon in question and such genera will correspond to species that lived at a certain time in the past and have not gone extinct. We adopted this logic and attempted to find such a cut. However, we were surprised to find that the time-points to define currently used genera are rather inconsistent. For instance, *Jemadia* diverged about 10 Mya and the two species placed in different genera: *Mysoria* [formerly *Metardaris*] *cosinga* diverged from *Mysoria* [formerly *Sarbia*] *xanthippe* about 2 Mya. While we may doubt absolute dates for these divergences, the 5x ratio between them is more confident.

Researchers agree that genera should correspond to major evolutionary groupings above species and below a subtribe. "Major" would mean groups separated by relatively longer branches and higher statistical support. Conversely, within the group, close to the time the group split into subgroups, statistical support could be lower, indicating rapid radiation. In the tribe Pyrrhopygini, there are 4 major groups (Fig. 1). Divergence within these 4 groups is quite consistent and dates to about 15 Mya. The groups are supported by some of the longest branches in the tree. Here, we call them subtribes. Three of these subtribes are new, described here in the main text.

However, these groups could be taken as genera. These would be rather broadly defined genera compared to current classification and would "sink" many currently used genera. Such a "lumper" treatment is listed in the SI Appendix. It groups species into genera that are broader than any genus currently used. Current treatment would be "splitters" treatment, and even though some genera, like *Metardaris*, diverged from others so recently that they clearly do not merit the status of a genus. On the other hand, some more diverse genera would need to be split and new genera need to be proposed. This "splitter" treatment corresponding to the divergence about 5 Mya is also given in SI Appendix. Attempting to find a middle ground between the two treatments, we can take those today's genera that are more diverse, and cut the tree about that level to see if the cut defines groups that may be considered major. *Jemadia* (excluding *fallax*) and *Pyrrhopyge* (sensu stricto) are such diverse genera, and cutting about that level indeed results in a meaningful classification (lime-colored cut in Fig. 1) that is adopted here (SI Appendix).

Which one of the three (broad, middle, or narrow) treatments is the best? It seems to be a matter of personal preference. Narrow approach results in many monotypic genera, which are not different from having a single name for a species, because they do not suggest any relatives. Therefore, such genera are of limited utility. Our phylogenetic tree strongly indicates that the broad genera (equal to the subtribes) are most meaningful in terms of standing out as truly prominent groups. However, we see the recent trend in the literature to split genera further rather than lump them. Therefore, although we like the broad treatment, it may not be readily accepted by researchers today. In addition to genera, we also offer subgeneric classification. Subgenera indicate evolutionary clusters below genus but above species. To emphasize these clusters, 10 new subgenera are described in the main text.

Morphological divergence and genomic similarity.

Pyrrhopyginae are masters of disguise. Analysis of phenotypes defines a limited number of patterns that recur in different evolutionary groups: (1) black with fiery abdomen tip and frequently head, (2) sinimustvalge *Jemadia* present in many genera, (3) dark metallic green or blue with some red on wings, (4) brown with dark lines and white spots and black with yellow stripes. While we see these recurring patterns, we do not observe features combined. For instance, there is no red abdomen tip in sinimustvalge-patterned skippers. Sticking to limited number of patterns is likely mimetic, although the mechanisms of such mimicry are not yet well understood.

We also see that there could be pronounced intra-species variation of wing patterns. A common feature of such variation is the presence or absence of a white band across the wings. Several species such as *Aspitha*

agenoria, *Gunayan rubricollis*, *Microceris* [formerly *Elbella*] *iphinous*, *Microceris* [formerly *Elbella*] *luteizona*, *Oxynetra* [formerly *Olafia*] *roscius* display polymorphism with banded and non-banded forms. The well-known transition between sergius (black wings, broad white hindwing margin crossed by black veins), hyperici (bluish-white spots on hindwing above, wide white at the base below), bixae (hindwing black above, narrower white below at the base) and phidias (wings solid black) forms of *Pyrrhopyge* recurring in many species suggests extreme plasticity of wing pattern in these skippers.

Finally, we note recurring divergence leading to unique wing patterns. Most prominent are *Microceris variicolor* and *Protelbella* [formerly *Ochropyge*] *ruficauda* which are so different from their closest relatives that these species were placed in monotypic genera before. In addition to wing patterns, these two species display rapid and profound divergence in genitalia that hindered their evolutionary closeness to their kin as revealed by genomic analysis.

References:

Talavera G, Lukhtanov VA, Pierce NE, Vila R. 2012 Establishing criteria for higher-level classification using molecular data: the systematics of *Polyommatus* blue butterflies (Lepidoptera, Lycaenidae). Cladistics 29, 166-192.

Methods

M1. Sample collection and genomic DNA extraction

We preserved different parts of the butterfly specimen for DNA extraction depending on the source and the condition of the sample. For freshly collected specimens, we removed the head of the specimen and preserve in alcohol for DNA extraction. If the head provided insufficient materials, we dissected the chest muscle. For old and dry samples from insect collections, we used either legs or a whole abdomen (dropped into lysis buffer for overnight incubation at 56 °C, and then transferred into 10% KOH for genitalia dissection) to extract genomic DNA with Macherey-Nagel (MN) NucleoSpin® tissue kit following the manufacturer's protocol. Genomic DNA was eluted in a total volume of 30-50 µl QIAGEN AE buffer, and the concentration of DNA was measured by Promega QuantiFluor® dsDNA System.

M2. Sequencing library preparation protocol

M2.1. Paired-end library preparation protocol

NEBNext® Ultra™ II DNA Library Prep Kit for Illumina® was used for paired-end library preparation. Starting Material is 5 - 250 ng of fragmented DNA.

A. DNA Fragmentation

Depending on the genomic DNA quality (as determined by gel electrophoresis), some of the genomic DNAs were fragmented using a Covaris focused ultrasonicator S2 or S220 to 400 bp according to manufacturer's instructions, and then purified with 1.8X AMPure XP beads. DNA samples from some old dry samples are already degraded with smears ranging from <50 bp to 500 bp did not go through fragmentation.

B. End Preparation

1. Mix the following components in a sterile nuclease-free tube.

End Prep Enzyme Mix	1.5 µl
End Repair Reaction Buffer (10X)	3.5 µl
Fragmented DNA	25 µl

2. Place in a thermocycler, with the heated lid on, and run the following program:
   20°C for 30 min
   65°C for 30 min
   Hold at 4°C

C. Adaptor Ligation

If DNA input is < 10 ng, dilute the NEBNext Adaptor for Illumina (provided at 15 µM) 10-fold in 10 mM Tris-HCl or 10 mM Tris-HCl with 10 mM NaCl to a final concentration of 1.5 µM, use immediately.

1. Add the following components directly to the End Prep reaction mixture and mix well.

NEBNext Adaptor for Illumina	2.5 µl
Blunt/TA Ligase Master Mix	15 µl
Ligation enhancer	0.5 µl

2. Incubate at 20°C for 15 minutes in a thermal cycler.

D. USER excision

1. Add 2.5 µl USER™ enzyme to the ligation mixture from previous step.
2. Mix well and incubate at 37°C for 20 minutes.

E. Cleanup of Adaptor-ligated DNA

1. Move AMPure XP Beads to room temperature for 20 min. Vortex beads to resuspend.
2. Add 1.2X resuspended AMPure XP Beads to the ligation reaction. Mix well.
   For samples less than 10 ng or smaller than 100 bp, we used 1.6X Ampure XP beads we prepared in-house with higher (30%) concentration of PEG.
3. Incubate for 10 minutes at room temperature.
4. Quickly spin the tube and place it on an appropriate magnetic stand to separate beads from supernatant. After the solution is clear (about 5 minutes), carefully remove and discard the supernatant.
5. Add 200 µl of 80% freshly prepared ethanol to the tube, resuspend well. Incubate at room temperature for 30 seconds, and move plate to magnetic rack wait till clear, then carefully remove and discard the supernatant.
6. Repeat Step 5 once.
7. Air dry the beads.
8. Remove the tube/plate from the magnet. Elute the beads twice with 17 µl TE buffer.
9. Mix well by pipetting up and down. Incubate for 5 minutes at 37°C.
10. Quickly spin the tube and place it on the magnetic stand.
11. After the solution is clear (about 5 minutes), transfer 15 µl of the elution to a new PCR well plate.
12. Elute a 2nd time with 10 µl, to a final volume of 25 µl.
13. Measure concentration using Promega QuantiFluor® dsDNA System with 1 µl.

F. PCR Enrichment of Adaptor Ligated DNA

1. Mix the following components in a sterile nuclease-free tube:

Adaptor Ligated DNA Fragments & H2O (with up to 24 ng DNA)	23 µl
Index Primer	1 µl
NEBNext Q5 Hot Start HiFi PCR Master Mix	25 µl
Universal PCR Primer	1 µl

2. PCR with the following conditions. We use 6 cycles if 24 ng template DNA is used, and we increase it with less amount of DNA.

CYCLE STEP	*TEMP*	*TIME*	*CYCLES*
Initial Denaturation	98°C	30 seconds	1
Denaturation	98°C	10 seconds	6–15*
Annealing/Extension	65°C	90 seconds	
Final Extension	65°C	5 minutes	1
Hold	4°C	∞	

G. Cleanup and size selection of PCR Amplification

1. Add water to adjust the final volume of each reaction product to 100 µl.
2. Vortex AMPure XP Beads to resuspend.
3. Add 0.625X of resuspended AMPure XP Beads to the PCR reactions. Mix well by pipetting up and down at least 10 times.
4. Incubate for 10 minutes at room temperature.
5. Quickly spin the tube and place on an appropriate magnetic stand to separate the beads from the supernatant. After the solution is clear (about 5 minutes), carefully transfer the supernatant containing your DNA to a new well plate. Discard the beads that contain the unwanted large fragments.
6. Add 0.375X resuspended AMPure XP Beads for the 2nd time to the supernatant, mix well and incubate for 10 minutes at room temperature.
7. Quickly spin the tube and place it on an appropriate magnetic stand to separate the beads from the supernatant. After the solution is clear (about 5 minutes), carefully remove and discard the supernatant that contains unwanted DNA (to the plate with first time beads). Be careful not to disturb the beads that contain the desired DNA targets (Caution: do not discard beads).
8. Add 200 µl of 80% freshly prepared ethanol to the tube while in the magnetic stand. Incubate at room temperature for 30 seconds, and then carefully remove and discard the supernatant.
9. Repeat Step 8 once.
10. Air dry the beads for 5 minutes. Caution: Do not overdry the beads. This may result in lower recovery of DNA target.
11. Elute the the beads with 20 µl of 10 mM Tris-HCI or 0.1 X TE. Mix well on a vortex mixer or by pipetting up and down. Incubate for 5 minutes at 37°C.
12. Quickly spin the tube and place it on a magnetic stand. After the solution is clear (about 5 minutes), transfer 18 µl to a new well plate.
13. Elute a 2nd time with 19 µl, to a final volume of 37 µl.
14. Measure libraries concentration with 2 µl.

F. Check library size on 2% E-gel with a 100bp ladder

M2.2. Preparation for sequencing on the Illumina Hiseq X ten platform

The concentration of each library was quantified using Promega Quantus™ fluorometer with Promega QuantiFluor® dsDNA system, and the library size was estimated using gel electrophoresis. These two measurements were used to estimate the molar concentration of each library. The relative volume of each library was determined by the needed fraction and the molar concentration of the library. We typically target 10X coverage for each Pyrrhopyginae skipper sample, which is 6 Gbp data. Libraries of samples are pre-pooled, and we used Hiseq X ten sequencing service from GENEWIZ, which typically produces 130 Gbp data per lane.

M3. Nuclear and mitochondrial protein-coding gene assembly and preparation of intron alignment

M3.1. Processing of sequencing reads

NGS reads were processed sequentially by AdapterRemoval software (version 1.5.4) [1] to remove reads contaminated by adapters and oligos used in the sequencing reactions, and to trim low-quality (quality score < 20) portions at both ends. Below is the command and parameters used for paired-end libraries:

> AdapterRemoval --file1 [R1.fq] --file2 [R2.fq] --basename [sampleID] --trimns --trimqualities --minquality 20 --pcr1 [Adaptor1] --pcr2 [Adaptor2] # [R1.fq] and [R2.fq] are the paired-end FASTQ format sequencing reads, [sampleID] is the sample identification number, [Adaptor1] and [Adaptor2] are the adapter sequences used for the sample.

M3.2. Preparation for protein-coding gene assembly

We had attempted to perform a BWA [2]-GATK [3]-based mapping assembling strategy as described in Cong et al. [4]. However, due to the long evolutionary distance between our target species and available reference genomes, such BWA-GATK-based approach results in poor-quality genome assemblies, most of which cover only 10% ~ 20% of the reference genome (data not shown). Since the protein-coding regions still tend to be more conserved and can be aligned better with the help of protein sequences, we limited ourselves to exons and assembled coding sequences in the genomes.

The increased sensitivity of the protein-based approach permitted a high-quality alignment among our samples from diverse groups. However, this approach might have problems when reads from different paralogs are mapped to a single protein. To avoid mapping of paralogous reads, we applied cutoffs of sequence identity of the mapped reads for each exon. The two available genomes, *Cecropterus lyciades* [5] and *Lerema accius* [6] were used to estimate these cutoffs. *Cecropterus* exons were used as reference and we prepared reference exon set and identity cutoffs as follows:

1. Exons of *Cecropterus* with length less than 11 aa and ones with shorter length but highly identical (sequence identity >=95%) to other long exons were removed from reference exon set. In total, 89863 exons were included in the reference exon set.

2. Run TBLASTN [7] to perform a search using the amino acid) sequences of exons in one species against the nucleotide sequences of exons in another species. We disabled the low complexity sequence filter in TBLASTN by '-seg no'.

3. In the TBLASTN result, we calculated the sequence identity and E-value to the query exon for each hit and identified the lowest E-value hits for every query. If the statistics of other hits are comparable (difference in sequence identity < 5% and difference in log(e-value) < 5), we would also include them into the best hit set of the query.

4. To remove False Positives among the best hits for each query exon, we applied several filters: (a) the hits have to show e-value < 0.001 or sequence identity to the query > 90%; (b) discard hits with identity to query less than 50%; (c) discard ambiguous hits, which are detected as the best hit to multiple query exons, or multiple locations of the same query exon.

M3.3. Protein-coding sequence assembly for phylogenetic study from sequencing reads

Briefly, we performed TBLASTN search using *Cecropterus* exons obtained in M3.2 as queries to search against reads of each sample. Reads were discarded if they are mapped to several exons with similar quality or their sequence identity to exons is less than cutoff obtained in M3.2. Next, pair-wise comparisons of reads were performed to remove reads that are divergent to majority of reads and then consensus polymorphism was taken for each position in exons. The following is the detailed procedure:

1. Transfer the FASTQ format into FASTA format and format it as BLAST database.
2. Perform TBLASTN search using *Cecropterus* exons as queries. In the search, we turned off the low complexity filter by '-seg no' and allowed more hits by '-max_target_seqs 50000000'.
3. Apply filters based on BLAST output statistics by requiring: (a) hit coverage >= 75%, (b) identity to the query >= 50%, and >= highest identity among reads from all samples – 10 %.
4. Utilize the alignment between two reference genomes in the section M3.2 to filter out reads with sequence identity less than that between *Cecropterus* and *Lerema* by more than 5% in the same region. If the corresponding region is not present in the *Cecropterus-Lerema* alignment, the full *Cecropterus-Lerema* alignment will be used to estimate the identity cutoff.
5. Filter out the ambiguous hits that are mapped to multiple query exons, or multiple locations of the same query exon.
6. Exons with coverage 2.5 times of median exon coverage were considered as repeats and were removed from final alignment.
7. Assemble the aligned reads for each specimen with the following procedure:
    a. Compute the dominant nucleotide at each position. If the frequency of the dominant nucleotide is less than 80%, we are not confident about whether the observed polymorphism is due to population diversity or data quality issues, and we mark them as potential bad positions.
    b. Check the enrichment of such potential bad positions using a 24bp sliding window. If there are more than 2 potential bad positions in a window, filter out this window.
    c. Compute the average read coverage of the exon and filter out exons whose sequencing depth is less than 1.5, to ensure that most of the exons should be supported by at least two sequencing reads.
8. Finally, we used the exon set defined in section M3.2 and concatenate the assembled exons into a single FASTA sequence for phylogenetic studies.

M3.4. Z-linked genes identification and alignment preparation

Because high conservation of gene content has been reported in Lepidoptera Z chromosome [8], we aligned *Cecropterus* exons using TBLASTN (-evalue 0.001 -seg no) to *Heliconius* genome [9] where Z chromosome sequence was known. We identified *Cecropterus* exons as Z-linked if their best TBLASTN hit was on *Heliconius* Z chromosome. Genes with more than 80% exons mapped to Z chromosome were considered Z-linked. The sequences of the Z-linked genes were concatenated for each specimen. Positions with more than 60% of gaps in the alignment were removed before phylogenetic analysis.

M3.5. Assembling mitochondrial protein-coding genes

We took the 13 mitochondrial proteins from *Cecropterus* mitogenome and assembled these sequences for all samples. The assembly strategy for mitochondrial genes is almost identical to that for nuclear genes, with a few exceptions:

1. In TBLASTN search, we specified '-db_gencode 5' to switch to the invertebrate mitochondrial codon table.
2. We increased the read coverage cutoff from 1.5 to 3, as mitochondrial genomes generally have much higher coverage than the nucleus genome.

Finally, we obtained the concatenated mitogenome consisting of 11,178 aligned positions for our samples.

M3.6. Intron alignment preparation

BWA-GATK-based mapping assembling strategy was performed as described in [4]. Briefly, we mapped sequencing reads of 121 samples to genome of *Cecropterus* by BWA and detected single-nucleotide polymorphisms (SNPs) using GATK [3]. Intron regions suggested by *Cecropterus* annotation were concatenated for each specimen. The positions with more than 40% of gaps were removed from the alignment.

M4. Phylogenetic analysis

M4.1. Maximum-likelihood phylogenetic analysis of nuclear/mitochondrial protein-coding regions, introns and Z-linked protein-coding regions

For a thorough phylogenetic analysis, we prepared several datasets: nuclear protein-coding regions, Z-linked protein-coding regions, intronic regions and mitochondrial protein-coding regions. RAxML [10] (model: GTRGAMMA) was used to build maximum-likelihood trees using these datasets. To evaluate the confidence of the nuclear tree, we split the concatenated alignment of nuclear protein-coding sequences into 100 partitions with about 0.1 million positions in each partition. We applied RAxML (-m GTRGAMMA) on these 100 partitions and produced a consensus tree using SumTrees (https://pythonhosted.org/DendroPy/programs/sumtrees.html) with -f0.0. The confidence of trees built from Z-linked protein-coding regions, introns and mitochondrial protein-coding regions were estimated by 100 bootstrap replicates of alignments.

M4.2. The BEAST tree and time calibration

We carried out time-calibration using BEAST v2.5.1 [12]. To minimize the effects of gaps on time calibration and meanwhile to increase the speed of running the tree, we ranked the positions in nuclear protein-coding region alignment by gap ratio from low to high and used first 15K positions with lowest gap ratio. Yule model [13] was selected as tree prior option. In the absence of Pyrrhopyginae fossils, time constraints were set based on dating estimates given in previous publications that also included taxa outside Pyrrhopyginae [14], and even outside of Hesperiidae [11]. We found species present in all these trees, measured the times estimated for their divergence in these publications, and set BEAST constraints based on these estimates. The BEAST input file was submitted to Dryad (https://doi.org/10.5061/dryad.q0sr5p5). In brief, we constrained the time estimates for the common

ancestors of the following pairs of taxa: *Myscelus-Pyrrhopyge* (31.6 Mya), *Apyrrothrix-Creonpyge* (10.6 Mya), *Apyrrothrix-Pyrrhopyge* (15.4 Mya), *Creonpyge-Mysoria ambigua* (18.5 Mya), *Microceris-Parelbella* (10.6 Mya), *Microceris-Pyrrhopyge* (21.7 Mya), and *Agara belti-Passova* (11.1 Mya). We selected these pairs to represent a widest time scale range (10 to 30 My) and to constrain various segments of the tree. The ages given were set to 3/4 of the ages in Sahoo et al. [14], the study that contains a time-calibrated tree with the largest number of Pyrrhopyginae taxa. We think that this adjustment makes time estimates more realistic, because it approximately matches the time estimates from Espeland et al. [11], a study based on broad sampling of all butterflies and thus expected to be more accurate. The scale 3/4 was computed based on *Heteropterus-Piruna* divergence estimated at 42 Mya in Sahoo et al. [14] and 33 Mya in Espeland et al. [11]. It is important to note, that while the precise time estimates may be prone to error (+/-10 My) and are expected to be refined in future with more taxa included and more fossils discovered, relative time estimates (i.e. ratios of branch lengths throughout the tree) are expected to be more accurate. Only these relative estimates and tree topology were important in suggesting the higher classification of Pyrrhopyginae. When setting the time constrains, monophyletic option was selected to ensure all child nodes of constrained nodes were always together during the sampling. MCMC chain length was set to 6,000,000. A maximum clade credibility (MCC) tree was constructed using the program TreeAnnotator with 10% burn-in.

Additionally, we performed time-calibration by re-scaling the RAxML trees by assuming a constant evolutionary rate for every branch and rescaled the branches to obtain constant length from the leaf to the root. The procedure was as follows:

1. We took the largest branch length from the root to the leaves as the target branch length. Every branch was rescaled to the target branch length (TBL).
2. From the root, we iteratively repeated the following steps to rescale each internal branch to the expected length:
    a. at an internal node, subtract the branch length from this node to the root from the TBL to obtain the targeted remaining branch length (TRBL)
    b. identify the best path to a leaf from current node as the path with the largest number of internal nodes and computed the branch length of this best path, namely, current remaining branch length (CRBL)
    c. compute the scaling factor as the ratio of TRBL vs. CRBL
    d. multiply the scaling factor with the branch length for each branch in the best path, and update the branch length
    e. go to the children nodes and repeat step (a) until reaching the tips of the tree.

Time axis was added to the tree based on fossil calibration carried out in recent studies [11], [14] as described in the first paragraph of this section.

M4.3. Coalescent-based estimation by Astral using nuclear protein coding regions

In addition to the maximal likelihood approach, we also performed coalescent-based species tree estimation using ASTRAL [15] (version 5.5.9). For each gene alignment, positions with gap ratio more than 60% was discarded. Next, gene alignments with less than 5 specimens were excluded from the following analysis. In total, 13579 gene trees were constructed by RAxML (-m GTRGAMMA) with bootstrap replicates 100 (-# 100) on individual gene alignments. The nodes with less than 10% support in each gene tree was contracted as suggested by Zhang et al. [15] and gene trees where two outgroup samples were not grouped together were excluded from ASTRAL analysis. The default settings of ASTRAL was used to summarize individual nuclear gene trees.

M4.4. TreeMix

To exclude the possibility that the introgression affected phylogenetic analysis, we used TreeMix v1.12 [16]. Given that large number of gaps may affect performance of the program, we selected specimen that has less than 50% of gaps in the concatenated alignment of nuclear protein-coding regions. The bi-allelic positions present in more than 60% of selected specimens were kept. The frequency of each allele at each position was counted in each specimen as input for the program. We ran TreeMix with the settings -k 5 -noss.

M4.5. Detection of diagnostic nucleotide characters

To support the phylogenetic groups, we detected the distinguishing nucleotide characters that were mutated and maintained in the groups. We would like to find the characters that are (a) conserved within the group, (b) conserved in the rest species outside the group, and (c) different between the group and the rest sequences. Some of our samples are of poor quality and contained lots of gaps in the final alignment. To avoid possible mis-identification due to the missing characters, we constrained our positions to filter out positions dominated by gaps. In addition, we also had more stringent gap thresholds for the sister groups to the group of interests, to ensure that the characters we found indeed can differentiate the group of interest and its sister group. Below is the detailed procedure.

1. Define the group of interest (group I), its sister group (group S), and remaining group (group R, excluding the outgroups used for rooting the tree).
2. Define good positions as those that are not gaps in 80% of the samples (excluding poor samples).
3. Among good positions, extract the positions that are 100% conserved and have no gaps within group I, and definite these positions as P1 set.
4. Among the P1 set, remove those positions where the conserved characters for group of interest also appeared in the rest samples (group S and group R), resulting in P2 set.
5. Among the P2 set, only take the positions where the character in the rest (group S and group R) was conserved in more than 80% of the samples, and different from the character in the group I, resulting in P3 set.
6. Among the P3 set, filter out the positions where any species in the sister groups has a gap.

References

[1] Lindgreen, S. 2012 AdapterRemoval: easy cleaning of next-generation sequencing reads. *BMC Res Notes* **5**, 337. (doi:10.1186/1756-0500-5-337).

[2] Li, H. & Durbin, R. 2010 Fast and accurate long-read alignment with Burrows-Wheeler transform. *Bioinformatics* **26**, 589-595. (doi:10.1093/bioinformatics/btp698).

[3] McKenna, A., Hanna, M., Banks, E., Sivachenko, A., Cibulskis, K., Kernytsky, A., Garimella, K., Altshuler, D., Gabriel, S., Daly, M., et al. 2010 The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res* **20**, 1297-1303. (doi:10.1101/gr.107524.110).

[4] Cong, Q., Shen, J., Warren, A.D., Borek, D., Otwinowski, Z. & Grishin, N.V. 2016 Speciation in Cloudless Sulphurs Gleaned from Complete Genomes. *Genome Biol Evol* **8**, 915-931. (doi:10.1093/gbe/evw045).

[5] Shen, J., Cong, Q., Borek, D., Otwinowski, Z. & Grishin, N.V. 2017 Complete Genome of *Achalarus lyciades*, The First Representative of the Eudaminae Subfamily of Skippers. *Curr Genomics* **18**, 366-374. (doi:10.2174/1389202918666170426113315).
[6] Cong, Q., Borek, D., Otwinowski, Z. & Grishin, N.V. 2015 Skipper genome sheds light on unique phenotypic traits and phylogeny. *BMC Genomics* **16**, 639. (doi:10.1186/s12864-015-1846-0).
[7] Altschul, S.F., Gish, W., Miller, W., Myers, E.W. & Lipman, D.J. 1990 Basic local alignment search tool. *J Mol Biol* **215**, 403-410. (doi:10.1016/S0022-2836(05)80360-2).
[8] Fraisse, C., Picard, M.A.L. & Vicoso, B. 2017 The deep conservation of the Lepidoptera Z chromosome suggests a non-canonical origin of the W. *Nat Commun* **8**, 1486. (doi:10.1038/s41467-017-01663-5).
[9] Heliconius Genome, C. 2012 Butterfly genome reveals promiscuous exchange of mimicry adaptations among species. *Nature* **487**, 94-98. (doi:10.1038/nature11041).
[10] Stamatakis, A. 2014 RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30**, 1312-1313. (doi:10.1093/bioinformatics/btu033).
[11] Espeland, M., Breinholt, J., Willmott, K.R., Warren, A.D., Vila, R., Toussaint, E.F.A., Maunsell, S.C., Aduse-Poku, K., Talavera, G., Eastwood, R., et al. 2018 A Comprehensive and Dated Phylogenomic Analysis of Butterflies. *Curr Biol* **28**, 770-778 e775. (doi:10.1016/j.cub.2018.01.061).
[12] Bouckaert, R., Heled, J., Kuhnert, D., Vaughan, T., Wu, C.H., Xie, D., Suchard, M.A., Rambaut, A. & Drummond, A.J. 2014 BEAST 2: a software platform for Bayesian evolutionary analysis. *PLoS Comput Biol* **10**, e1003537. (doi:10.1371/journal.pcbi.1003537).
[13] Yule, G.U. 1925 II.—A mathematical theory of evolution, based on the conclusions of Dr. JC Willis, FR S. *Phil. Trans. R. Soc. Lond. B* **213**, 21-87.
[14] Sahoo, R.K., Warren, A.D., Collins, S.C. & Kodandaramaiah, U. 2017 Hostplant change and paleoclimatic events explain diversification shifts in skipper butterflies (Family: Hesperiidae). *BMC Evol Biol* **17**, 174. (doi:10.1186/s12862-017-1016-x).
[15] Zhang, C., Rabiee, M., Sayyari, E. & Mirarab, S. 2018 ASTRAL-III: polynomial time species tree reconstruction from partially resolved gene trees. *BMC Bioinformatics* **19**, 153. (doi:10.1186/s12859-018-2129-y).
[16] Pickrell, J.K. & Pritchard, J.K. 2012 Inference of population splits and mixtures from genome-wide allele frequency data. *PLoS Genet* **8**, e1002967. (doi:10.1371/journal.pgen.1002967).

Table S1. Data for 119 sequenced Pyrrhopyginae specimens and 2 outgroups

#	DNA Voucher	Taxon name	Ty	Sex	Locality	Elevation	GPS	Collectors	Date	Collection	Genitalia No.	Collection No.
1	NVG-17093H04	***Azonax typhaon***		M	French Guiana: nr. Cayenne, Montagne aux chevaux (Galion)		4.7250, -52.4083	Jean-Yves Gallard	7-Apr-1991	USNM		
2	NVG-18022F11	***Zonia zonia panamensis***	HT	M	Panama: Canal Zone, Madden Forest Preserve			Stan S. Nicolay	6-Feb-1968	AMNH	H379	
3	NVG-17093G06	***Granila paseas***		F	Brazil: Rio de Janeiro, Tijuca			Collection W. Schaus	N/A (old)	USNM		
4	NVG-17093G04	***Aspitha aspitha rufescens***		M	Brazil: Rondonia, 62 km S Ariquemes, Fazenda Rancho Grande	165 m	-10.53, -62.80	Ron Leuschner	29-Sep-10-Oct-1992	USNM		USNMENT 00894724
5	11-BOA-15607C08	***Aspitha aspitha aspitha***		F	Guyana: Upper Takutu-Upper Essequibo (Region 9), Kanuku Mts., Nappu Creek	150-300 m	3.3450, -59.5700	S. Fratello, R. Hanner, S. Hendricks, R. Williams	21-Feb-10-Mar-1999	USNM		USNMENT 00233032
6	NVG-18022F06	***Aspitha bassleri***	HT	M	Peru: San Martin, Achinamiza				11-Sep-1927	AMNH	G1408	
7	NVG-17108D03	***Aspitha agenoria agenoria***		M	Peru: Huanuco Region, 15 mi S Tingo Maria, Chinchavito	550 m			24-Jun-1982	LACM		
8	NVG-17093G09	***Myscelus nobilis***		M	Brazil: Rondonia, 8 km N Caculandia		-10.50, -62.87	D. H. Ahrenholz	5-Nov-1989	USNM		USNMENT 00894703
9	NVG-17094F07	***Myscelus amystis hages***		M	Costa Rica: Guanacaste Prov., ACG, Sector Del Oro, Quebrada Suamposa	290 m	11.02364, -85.49139	Lucia Rios	eclosed on 13-Mar-2005	USNM		05-SRNP-20512
10	NVG-17093F11	***Agara belti belti***		M	Costa Rica: Alajuela Prov., ACG, Sector Rincon Rain Forest, Sendero Albergue Crater	980 m	10.84886, -85.32810	Carolina Cano	eclosed on 19-Sep-2009	USNM		09-SRNP-4464
11	NVG-17094F06	***Agara michaeli***		F	Costa Rica: Alajuela Prov., ACG, Sector San Cristobal, Sendero Carmona	670 m	10.87621, -85.38632	Carolina Cano	eclosed on 14-Jun-2008	USNM		08-SRNP-1740
12	NVG-18022F09	***Passova nigrocephala***	HT	M	Colombia				prior to 1934	AMNH	G419	
13	NVG-15095D07	***Passova ganymedes ganymedes***	HT	M	Colombia				1925	CMNH	Slide No. 1957	
14	NVG-17094H03	***Passova gellias***		F	Costa Rica: Alajuela Prov., ACG, Sector San Cristobal, Sendero Carmona	670 m	10.87621, -85.38632	Carolina Cano	eclosed on 16-Nov-2013	USNM		13-SRNP-6248
15	NVG-17093G10	***Passova passova rudex***		M	Ecuador: Napo Prov., 4 km Tena-Pano Rd.	600 m	-1.03, -77.83	Stan S. Nicolay	28-Sep-1990	USNM		USNMENT 00894708
16	NVG-15095D06	***Pyrrhopyge cressoni***	HT	M	Bolivia: Santa Cruz, 75 km NW of Buenavista	450 m		Jose Steinbach	1926-1927	CMNH	Slide No. 1978	
17	NVG-17094H06	***Pyrrhopyge zenodorus***		M	Costa Rica: Alajuela Prov., ACG, Sector San Cristobal, Puente Palma	460 m	10.9163, -85.37869	Elda Araya	eclosed on 23-Apr-2015	USNM		15-SRNP-296
18	NVG-17094C07	***Pyrrhopyge evansi evansi***		M	Panama: Veraguas, Isla Coiba, Represa		7.50, -81.70	G. B. Small	25-Feb-1981	USNM		USNMENT 00894891
19	NVG-17094B11	***Pyrrhopyge phidias bixae***		M	Brazil: Para, Obidos				Nov-1962	USNM	V38 S. S. Nicolay	USNMENT 00894883
20	NVG-18022G08	***Pyrrhopyge phidias phidias*** (=***williamsi***)	HT	M	Peru				prior to 1931	AMNH	G423	
21	NVG-18022E11	***Pyrrhopyge proculus lina***	HT	M	Brazil: Para			A. M. Moss	prior to 1947	AMNH	G1812	
22	NVG-18022F07	***Pyrrhopyge proculus draudti***	HT	M	Bolivia: Santa Cruz				prior to 1931	AMNH		
23	NVG-17094C05	***Pyrrhopyge aziza lexos***		M	Guyana: Iwokrama Mts, Iwokrama Rainforest Reserve	750-950 m	4.3303, -58.7985	S. Fratello	28-Mar-1-Apr-2001	USNM		USNMENT 00179063
24	NVG-18022F05	***Pyrrhopyge aziza attis***	HT	M	Bolivia: Santa Cruz				prior to 1931	AMNH	G451	
25	NVG-18022F10	***Pyrrhopyge thericles orientis***	HT	M	Brazil: Para			A. M. Moss	prior to 1947	AMNH	G1813	
26	NVG-18022G02	***Pyrrhopyge thericles pseudophidias***	HT	M	Colombia: Muzo				prior to 1931	AMNH	G433	
27	NVG-18022G03	***Pyrrhopyge thericles rileyi***	HT	M	Bolivia: Santa Cruz				prior to 1931	AMNH	G453	
28	NVG-15111B05	***Pyrrhopyge thericles rileyi*** (AT of =***bolius***)		F	Bolivia: Dept. Santa Cruz, Buena Vista	400 m			prior to 1947	AMNH		
29	NVG-18022F12	***Pyrrhopyge amythaon peron***	HT	M	Peru: Iquitos				16-Feb-1932	AMNH	G687	
30	NVG-18022F04	***Pyrrhopyge amythaon amythaon***	HT	M	Brazil: Pernambuco				prior to 1931	AMNH	G1179	
31	NVG-15111B06	***Pyrrhopyge sergius ganus***	HT	M	Guyana			C. L. Pollard	prior to 1947	AMNH	G1262	
32	NVG-15111B04	***Pyrrhopyge sergius josephina*** (=***bolius***)	HT	M	Bolivia: R. Chimato	580 m			12-Apr-1926	AMNH	G1720	
33	NVG-15111B07	***Pyrrhopyge sergius josephina*** (=***mina***)	HT	M	Bolivia				prior to 1931	AMNH	G416	
34	NVG-15111B03	***Pyrrhopyge sergius andronicus***	HT	M	Ecuador: Macas				prior to 1931	AMNH	G383	
35	NVG-17094C03	***Pyrrhopyge sergius sergius***		M	Peru: Cuzco, Cosnipara Rd., San Pedro Lodge	1450 m		Steve Kinyon	6-Dec-2012	USNM		USNMENT 00894898
36	NVG-15095D08	***Pyrrhopyge guianae***	HT	M	French Guiana				before 1932	CMNH		
37	NVG-17094C04	***Pyrrhopyge pelota***		M	Bolivia: Beni, 40 km E San Borja Estacion Biologica Beni, Palm Camp			M. G. Pogue	11-Sep-1987	USNM		USNMENT 00894879
38	NVG-17094C12	***Pyrrhopyge kelita***		M	Peru: Cuzco, Mirador Cosnipata Rd.	1700 m		Steve Kinyon	5-Nov-2012	USNM		USNMENT 00894866
39	NVG-17094C08	***Pyrrhopyge papius papius***		M	Peru: San Martin, Rio Serranoyacu	1800 m	-5.667, -77.750	Robert K. Robbins & Gerardo Lamas	11-Nov-1998	USNM		USNMENT 00894901
40	NVG-18022G05	***Pyrrhopyge melanomerus*** (=***tarapotoensis***)	HT	M	Peru: Tarapoto				prior to 1931	AMNH	G424	
41	NVG-17094C01	***Pyrrhopyge hadassa pseudohadassa***		M	Peru: Cuzco, Cosnipata Valley	1375 m		Steve Kinyon	1-Oct-2014	USNM		USNMENT 00894877
42	NVG-17094C11	***Pyrrhopyge creona***		M	Peru: Amazonas, Abra Pardo Miguel	2200 m	-5.70, -77.80	Robert K. Robbins & Gerardo Lamas	11-Jun-2000	USNM		USNMENT 00894876
43	NVG-17094F05	***Pyrrhopyge telassa croceimargo***		M	Peru: Cuzco, Cosnipata Rd., San Pedro Lodge	1450 m		Steve Kinyon	11-Feb-2011	USNM		USNMENT 00894765
44	NVG-18022G04	***Pyrrhopyge schausi***	HT	M	Ecuador				prior to 1931	AMNH		
45	NVG-17094G01	***Pyrrhopyge haemon***		F	Costa Rica: ACG				2002	USNM		02-SRNP-35242
46	NVG-17093F09	***Pyrrhopyge crida***		M	Costa Rica: Guanacaste Prov., ACG, Sector Del Oro, Monte Cristo	525 m	11.01373, -85.42531	Roster Moraga	eclosed on 04-Apr-2009	USNM		09-SRNP-20790
47	NVG-18022G06	***Pyrrhopyge tatei***	HT	M	Venezuela: Provisional Camp, Mt. Duida				12-Dec-1928	AMNH	G1177	
48	OM34.748	***Gunayan rhacia***		M	Brazil: Santa Catarina, Joinville			O. H. H. Mielke	15-Feb-1981	OM-DZUP		
49	NVG-17093H12	***Gunayan rubricollis***		M	Peru: Madre de Dios, Rio La Torre, Tambopata National Reserve			D. H. Ahrenholz	5-Oct-1986	USNM		USNMENT 00894904
50	NVG-18022E09	***Gunayan rhacia*** (=***flemingi***)	HT	M	Venezuela: Caripito			Fleming	29-Jul-1942	AMNH	G1931	
51	NVG-17094A04	***Yanguna cometes cometides***		M	Peru: Madre de Dios, Tambopata National Reserve	300 m	-12.83, -69.28	D. H. Ahrenholz	6-Oct-1986	USNM		USNMENT 00894884
52	NVG-15096B06	***Yanguna thelersa*** (=***blossomiae***)	HT	F	Ecuador: Huigra	1200 m		W. J. Coxey	May-1930	CMNH		
53	NVG-17093F08	***Yanguna cosyra***		M	Costa Rica: Guanacaste Prov., ACG, Sector Pitilla, Sendero Orosilito	900 m	10.98332, -85.43623	Manuel Rios	eclosed on 01-Oct-2008	USNM		08-SRNP-31712
54	NVG-17094A07	***Yanguna spatiosa spatiosa***		M	Ecuador: Zamora-Chinchipe, 24 km Zamora-Loja Rd., Quebrada San Ramon	1700 m	-3.9693, -79.0625	David H. Ahrenholz	15-Jan-2002	USNM		USNMENT 00894887
55	NVG-15111F08	***Apyrrothrix (Apyrrothrix) araxes arizonae***		M	Mexico: Sonora, appr. 18 mi W Cananea			Peter Hubbell	26-Aug-1969	AMNH		
56	NVG-15111F01	***Apyrrothrix (Apyrrothrix) araxes araxes***		M	Mexico: Hidalgo, Cuesta Colorado	2600 m		William H. Howe	2-Sep-1978	AMNH		
57	NVG-18022G01	***Apyrrothrix (Melanopyge) mulleri*** (=***plancartei***)	HT	M	Mexico: Veracruz, Presidio				prior to 1941	AMNH		
58	NVG-18022E10	***Apyrrothrix (Melanopyge) hoffmanni***	HT	M	Mexico: Tabasco, Tenosique			E. C. Welling	3-Sep-1962	AMNH		
59	NVG-17093F05	***Apyrrothrix (Melanopyge)*** [n. sp.] "Burns01"		M	Costa Rica: Alajuela Prov., ACG, Sector San Cristobal, Sendero Vivero	730 m	10.86739, -85.38744	Carolina Cano	eclosed on 03-Sep-2014	USNM		14-SRNP-2944
60	NVG-2083	***Apyrrothrix (Melanopyge) maculosa***		F	Panama: Panama, Gamboa, Pipeline road	75 m	9.12717, -79.71483	John R. MacDonald	2-Jun-2012	MEM		
61	NVG-15096A12	***Apyrrothrix (Melanopyge) sangaris***	HT	M	Colombia: Magdalena, Sierra San Lorenzo, Hacienda Cincinnati	1400 m		Rehn and Hebard	23-Jul-1920	CMNH		
62	NVG-17093H02	***Apyrrothrix (Chalypyge) chalybea chloris***		F	Honduras: Zamorano				12-Aug-1957	USNM		USNMENT 00894713
63	NVG-17093H03	***Apyrrothrix (Chalypyge) hygieia***		M	Ecuador: Esmeraldas, Rio Chuchuvi, km 12.5 Lita-San Lorenzo Rd.	800-900 m	0.8835, -78.5150	I. Aldas	1-May-2002	USNM		USNMENT 00894709
64	NVG-17094F12	***Apyrrothrix (Aesculapyge) aesculapus***		F	Costa Rica: Guanacaste Prov., ACG, Sector Cacao, Sendero Cima	1460 m	10.93328, -85.45729	Manuel Pereira	eclosed on 18-Apr-2011	USNM		10-SRNP-35667
65	NVG-17093F06	***Creonpyge creon creon***		M	Costa Rica: Guanacaste Prov., ACG, Sector Santa Maria, Santa Maria	840 m	10.75772, -85.30598	Calixto Moraga	eclosed on 13-Jun-2015	USNM		15-SRNP-30170
66	NVG-17093H06	***Jonaspyge jonas***		M	Mexico: Oaxaca, Sierra Juarez, Gulf slope	1800-2100 m		John Kemner	2-Aug-1991	USNM		USNMENT 00894867
67	NVG-15111B08	***Jonaspyge tzotzili***	HT	F	Mexico: Chiapas, Ocozingo			T. Escalante	Jul-1942	AMNH		
68	OM11.938	***Ardaris aerata***		M	Colombia: Sierra Nevada de Santa Marta			A. Schultze	19-Mar-1928	OM-DZUP		
69	NVG-14106H10	***Ardaris eximia***		F	Venezuela: Merida, Paramo La Culata	3200-3400 m		C. Bordon	6-Aug-1984	USNM		
70	NVG-17109E07	***Ardaris sela sela***		M	Colombia: Prov. Santa Maria, Boyaca				10-Mar-1983	LACM		
71	NVG-17094D05	***Ardaris sela periphema***		M	Peru: Cuzco, Cosnipata Valley, San Pedro vicinity	1373 m	-13.0583, -71.5344	Brian Harris	6-Feb-2011	USNM		USNMENT 00894768
72	NVG-17109E06	***Ardaris pityusa***		M	no data				N/A	LACM		
73	NVG-17094D07	***Ardaris minthe***		M	Peru: San Martin, Puente Aguas Verdes	950 m	-5.683, -77.650	Robert K. Robbins & Gerardo Lamas	10-Nov-1998	USNM		USNMENT 00894770
74	NVG-17094D10	***Mysoria (Sarbia) cosinga***		M	Peru: Cuzco, Quebrada. Chusa, km 88, Paucartambo/Acjanaco Rd.	3150 m		S. Kinyon	3-Nov-2016	USNM		USNMENT 00894773
75	NVG-17094E01	***Mysoria (Sarbia) xanthippe spixii***		F	Brazil: Santa Catarina, Sao Bento do Sul, Rio Vermelho	800 m		O. H. H. Mielke	21-Jan-1973	USNM		USNMENT 00894776
76	NVG-17093F04	***Mysoria (Mysarbia) sejanus stolli***		M	Costa Rica: Guanacaste Prov., ACG, Sector Pitilla, Sendero Mismo	680 m	10.98758, -85.41967	Petrona Rios	11-Feb-2010	USNM		10-SRNP-30468
77	NVG-17094E02	***Mysoria (Sarbiena) catomelaena***		M	Brazil: Mato Grosso, Serra do Cipo	1250 m	-19.267, -43.583	Robert K. Robbins & Becker	17-Apr-1991	USNM		USNMENT 00894777
78	NVG-17094H07	***Mysoria (Mysoria) ambigua***		F	Costa Rica: Guanacaste Prov., ACG, Sector Mundo Nuevo, Quebrada Tibio Perla	330 m	10.76261, -85.42979	Jose Cortez	eclosed on 22-Oct-2013	USNM		13-SRNP-56544
79	NVG-17094E10	***Mysoria (Mysoria) barcastus barcastus***		M	Suriname: Commewijne De Nieuwe Grond			Olle Pellmyr	6-Feb-1982	USNM		USNMENT 00894755
80	NVG-18022G09	***Mysoria (Mysoria) affinis*** (=***wilsoni***)	HT	M	Mexico: Guerrero, Mexcala			Kent Wilson	22-Jul-1956	AMNH		
81	NVG-17094E05	***Mysoria (Amysoria) galgala***		M	Venezuela: Coniedes, San Carlos Bridge	300 m		D. H. Ahrenholz	8-Sep-2003	USNM		USNMENT 00894750
82	NVG-17094E07	***Mimoniades (Amenis) pionia pionia***		M	Bolivia: Chuguisace Prov., 30 km SE Carandaity			Stephen C. Bromley	Sep-1957	USNM		USNMENT 00894752
83	NVG-17094D03	***Mimoniades (Mahotis) versicolor eupheme***		M	Peru: Cuzco, Cosnipata Valley, San Pedro Lodge	1375 m		Steve Kinyon	20-Oct-2013	USNM		USNMENT 00894881
84	NVG-17094D04	***Mimoniades (Mahotis) nurscia nurscia***		M	Ecuador: Morona-Santiago, Rio Abanico	1600 m	-2.2617, -78.2033	I. Aldas	16-Oct-2002	USNM		USNMENT 00894855

85	NVG-15096A11	***Mimoniades (Mahotis) nurscia amans***	HT		Colombia: Neiva				before 1920	CMNH		
86	NVG-17094D02	***Mimoniades (Mimoniades) ocyalus***		M	Brazil: Santa Catarina, Sao Bento do Sul, Rio Vermelho	850 m		Miers	11-Feb-1991	USNM		USNMENT 00894896
87	NVG-17109E05	***Mimoniades (Mimadia) fallax***		M	Ecuador: Sucumbios Pr., Jct. Rd. Lago Agrio to Puerto El Carmen de Putamayo, Rio Cuyabeno	250 m	-0.56, -76.32	G. Kareofelas & C. Witham	18-Nov-15-Dec-1993	LACM		
88	NVG-17109E03	***Jemadia (Jember) scomber***		M	Peru: Huanuco Department, Tingo Maria				Feb-1984	LACM		
89	NVG-17094H02	***Jemadia (Jemadia) pseudognetus***		M	Costa Rica: Alajuela Prov., ACG, Brasilia, Moga	320 m	11.01227, -85.34929	Duvalier Briceno	eclosed on 12-Sep-2008	USNM		08-SRNP-65423
90	NVG-17094B09	***Jemadia (Jemadia) hospita hospita***		M	Ecuador: Pastaza/Napo, Rio Anzu	700 m	-1.33, -78.00	Velastiqui	2-Jan-1969	USNM		USNMENT 00894862
91	NVG-17094H01	***Jemadia (Jemasonia) suekentonmiller***	PT	M	Costa Rica: Guanacaste Prov., ACG, Sector Pitilla, Sendero Laguna	680 m	10.9888, -85.42336	Manuel Rios	eclosed on 26-Sep-2005	USNM		05-SRNP-31969
92	NVG-15029A07	***Nosphistia zonara*** (=***perplexus***)	?ST	M	Peru: Chanchamayo			Thamm	1885	ZMHB		
93	NVG-17094F02	***Croniades pieria pieria***		M	Brazil: Para, ca 60 km S Altamira, Rio Xingu Camp		-3.65, -52.37	P. Spangler & O. Flint	2-8-Oct-1986	USNM		USNMENT 00894759
94	NVG-17094F01	***Croniades pieria auraria***		M	Peru: Cuzco, Cosnipata Valley, Quebrada Santa Isabel	1194 m		Steve Kinyon	24-Oct-2016	USNM		USNMENT 00894758
95	OM33.661	***Protelbella (Ochropyge) ruficauda***		M	Brazil: Santa Catarina, Joinville				20-Dec-1992	OM-DZUP		
96	NVG-17094B03	***Protelbella (Protelbella) alburna alburna***		M	Bolivia: Cochabamba, Chapare, Rio Cristal Mayo	600 m		Jorge Kesselring	3-Jan-1958	USNM		USNMENT 00894698
97	NVG-17094F03	***Parelbella (Pseudocroniades) machaon machaon***		F	Brazil: Parana, Guaratuba, Castelhanos	750-800 m	-25.833, -49.017	Mielke, Robbins & Caldas	18-Mar-1995	USNM		USNMENT 00894760
98	NVG-17094G12	***Parelbella (Parelbella) macleannani***		F	Costa Rica: Alajuela Prov., ACG, Sector San Cristobal, Finca San Gabriel	645 m	10.87766, -85.39343	Osvaldo Espinoza	eclosed on 19-Sep-2013	USNM		13-SRNP-3787
99	NVG-17094B02	***Parelbella (Parelbella) ahira ahira***		M	Ecuador: Succumbios, Cerro Lumbaqui Norte	980 m	0.0283, -77.3203	David H. Ahrenholz	3-Jan-2002	USNM		USNMENT 00894723
100	NVG-17094B01	***Parelbella (Parelbella) polyzona***		M	Brazil: Santa Catarina, Joinville	0-200 m	-26.317, -48.883	H. Miers	6-Jan-1990	USNM		USNMENT 00894733
101	NVG-17094G04	***Microceris (Merobella) merops***		M	Costa Rica: Alajuela Prov., ACG, Sector Rincon Rain Forest, Rio Francia Arriba	400 m	10.89666, -85.29003	Anabelle Cordoba	eclosed on 18-Feb-2012	USNM		11-SRNP-44852
102	NVG-17094G07	***Microceris (Blubella) patrobas patrobas***		M	Costa Rica: Guanacaste Prov., ACG, Sector Pitilla, Medrano	380 m	11.01602, -85.38053	Ricardo Calero	eclosed on 26-Oct-2013	USNM		13-SRNP-71619
103	NVG-17094G05	***Microceris (Blubella) miodesmiata***		M	Costa Rica: Guanacaste Prov., ACG, Sector Pitilla, Medrano	380 m	11.01602, -85.38053	Ricardo Calero	eclosed on 19-Aug-2012	USNM	NVG131102-93	12-SRNP-71305
104	NVG-18041C07	***Microceris (Blubella) etna***		M	Peru: San Martin, 40 km SW Juanjuí	2000 m		Coll. Fritz Konig	Dec-1999	Brockmann		
105	NVG-18022F03	***Microceris (Blubella) adonis***	HT	M	Paraguay: Villarica				Nov, prior to 1931	AMNH		
106	NVG-18022E12	***Microceris (Apatiella) mariae mariae***	HT	M	Brazil: Rio Grande do Sul				prior to 1931	AMNH		
107	NVG-17094D01	***Microceris (Apatiella) iphinous***		M	Brazil: Rio de Janeiro, Casimiro de Abreu (Aldeia Velha)	200-400 m	-22.650, -42.383	Astrid Caldas & students	24-Jan-1995	USNM		USNMENT 00894871
108	NVG-18022G07	***Microceris (Microceris) theseus***	HT	M	Brazil				prior to 1934	AMNH	G531	
109	NVG-17093F10	***Microceris (Microceris) scylla***		M	Costa Rica: Guanacaste Prov., ACG, Sector El Hacha, La Guitarra	355 m	10.99378, -85.52108	Elieth Cantillano	eclosed on 13-Dec-2008	USNM		08-SRNP-23853
110	NVG-15095D05	***Microceris (Microceris) viriditas viriditas*** (=***carriae***)	HT	M	Paraguay: Sapucay			Rodolfo Heinrich	11-Feb-1925	CMNH	Slide No. 1958	
111	NVG-17094E12	***Microceris (Microceris) variicolor***		M	Brazil: Mato Grosso			Coll. B. Neumogen	N/A (old)	USNM		USNMENT 00894757
112	NVG-17093F12	***Oxynetra (Olafia) roscius roscius***		M	Brazil: Santa Catarina			Fritz Hoffmann	28-Jan-1956	USNM		USNMENT 00894737
113	OM55.572	***Oxynetra (Olafia) roscius flavomaculata***		M	Brazil: Santa Catarina, Bento do Sul, Rio Vermelho	850 m		Rank	4-Feb-2002	OM-DZUP		
114	NVG-15029A10	***Oxynetra (Olafia) roscius iphimedia*** (=***erythrosoma***)	ST	M	Brazil: Sao Paulo				prior to 1886	ZMHB		
115	NVG-14113A02	***Oxynetra (Dis) aureopecta***	HT	M	Mexico: Hidalgo, Puerto del Cabal	1020 m	21.167, -98.917	William H. Howe	8-Sep-1987	LACM		
116	NVG-2078	***Oxynetra (Dis) hopfferi***		M	Panama: Chiriqui, Santa Clara, Finca Hartmann	1540 m	8.84300, -82.76219	Rafael Munoz	11-Aug-2010	MEM		
117	NVG-17095B07	***Oxynetra (Dis) stangelandi***	PT	F	Costa Rica: Guanacaste Prov., ACG, Sector Cacao, Sendero Derrumbe	1220 m	10.92918, -85.46426	Harry Ramirez	eclosed on 19-Sep-2002	USNM		02-SRNP-23285
118	NVG-14112G09	***Oxynetra (Oxynetra) semihyalina***		M	Peru: Tingo Maria				N/A (recent)	MGCL		
119	NVG-14112G06	***Oxynetra (Oxynetra) confusa***		M	Peru: Tingo Maria				N/A (recent)	MGCL		
120	NVG-7993	***Celaenorrhinus syllius***		M	Ecuador: Sucumbios, Cerro Lumbaqui Norte	950 m	0.02833, -77.32033	J. P. W. Hall & M. A. Solis	1-3-Jan-2002	USNM	NVG170207-78	USNMENT 01321833
121	NVG-7335	***Tagiades gana meetana***		M	Myanmar: Rakhine Div., GwaTwsp Ye Phya, Chaung			Aung Gyi	2-9-May-2003	USNM	NVG161105-08	USNMENT 01321280

	Collection abbreviations
AMNH	American Museum of Natural History, New York, NY, USA
Brockmann	Research collection of Ernst Brockmann, Lich, Germany
CMNH	Carnegie Museum of Natural History, Pittsburgh, PA, USA
LACM	Los Angeles County Museum of Natural History, Los Angeles, CA
MEM	Mississippi Entomological Museum, Mississippi State University, MS, USA
MGCL	McGuire Center for Lepidoptera and Biodiversity, Gainesville, FL, USA
OM-DZUP	Research collection of Olaf H. H. Mielke, Curitiba, Brazil
USNM	National Museum of Natural History, Smithsonian Institution, Washington, DC, USA
ZMHB	Museum für Naturkunde, Berlin, Germany

Supplemental Figures

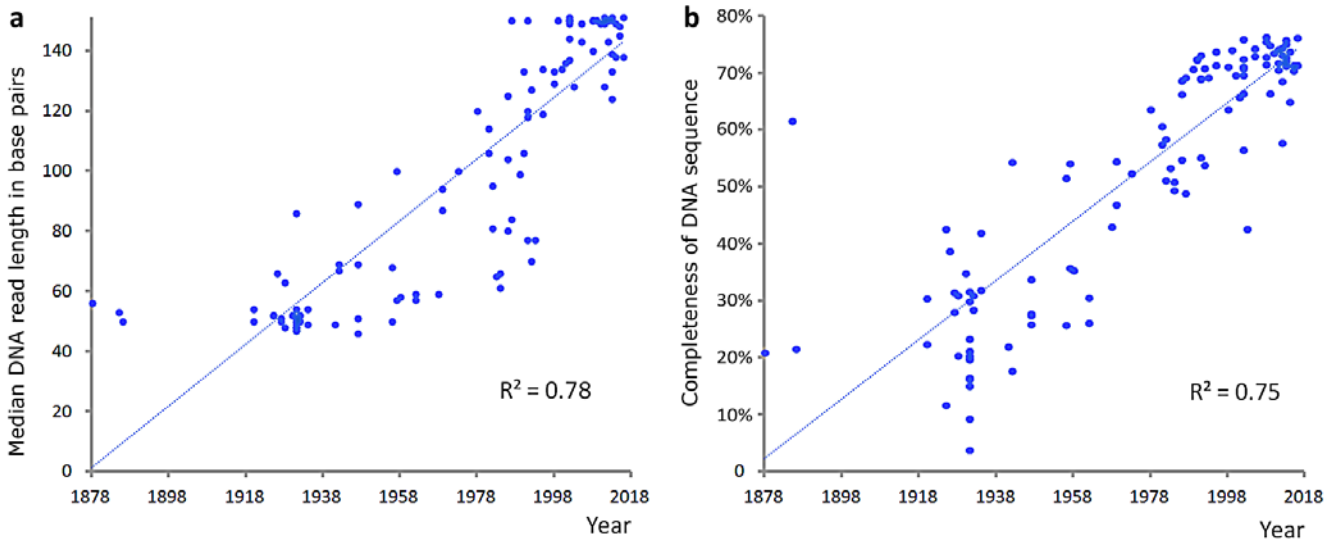

Figure S1. Specimen age and sequence quality. Each point represents a specimen. Collection year was not routinely recorded for specimens collected about a century ago. Because they were type specimens, they should have been collected prior to the publication of their description. Thus, the year used represents the latest year a specimen could have been collected. **a.** Correlation between median length of a sequence read (in base pairs) and a year no later than which it was collected. **b.** Correlation between genomic completeness (fraction) and a year no later than which it was collected. A trend line and the square of the correlation coefficient (R^2) are shown on the plots.

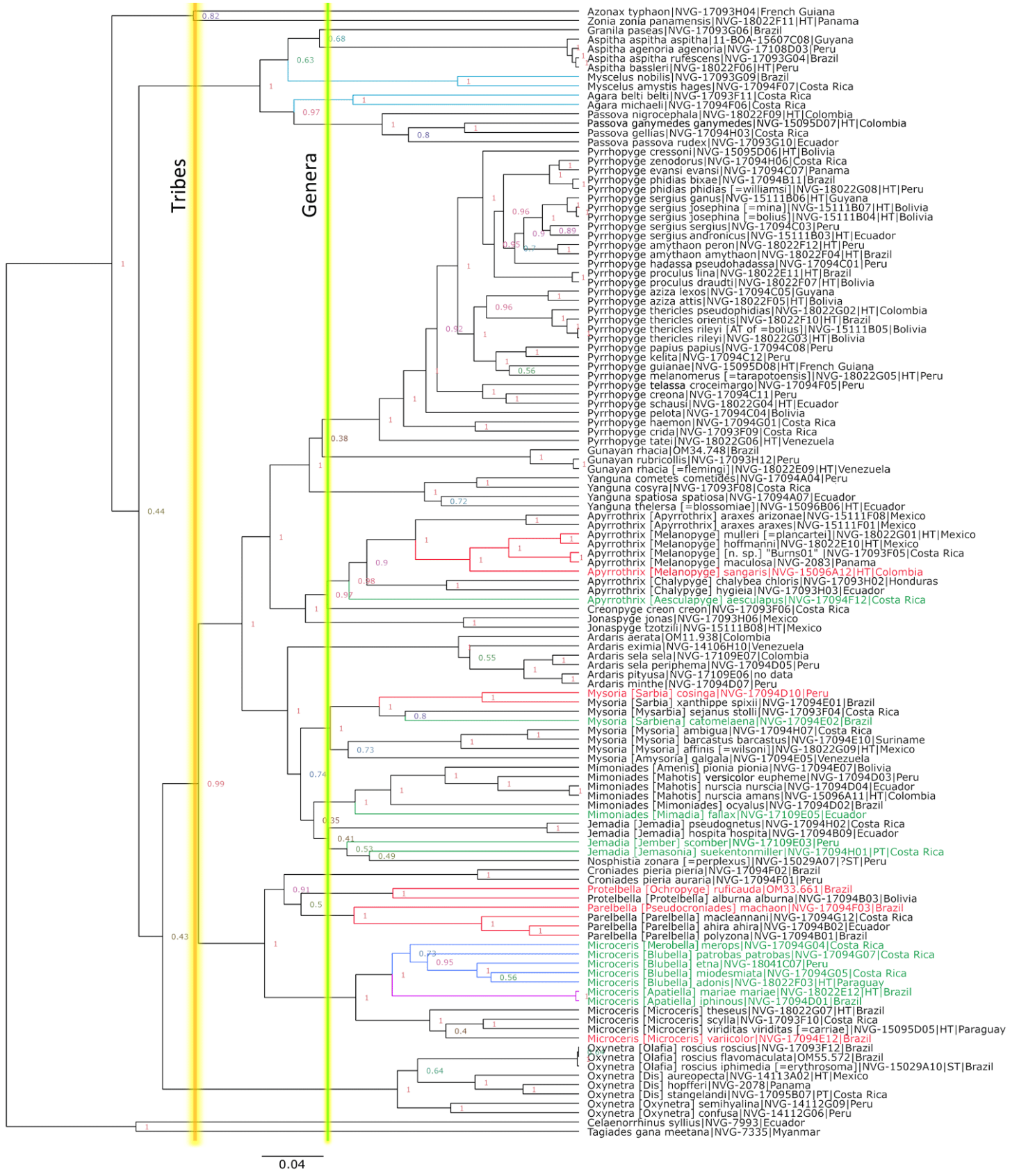

Figure S2. Time-calibrated tree of Pyrrhopyginae constructed from coding regions in mitochondrial genomes. Names in red show phenotypically diverged close relatives of species they were not associated with before. The clade joining them is shown in red. Names in green are new subgenera. Bootstrap values are shown by nodes. Branches in polyphyletic genus *Myscelus* (here split into 2 genera) are colored cyan.

Figure S3. Maximum likelihood phylogenetic tree constructed from intronic regions. Bootstrap values are shown by nodes.

Figure S4. Maximum likelihood phylogenetic tree constructed from Z-linked protein-coding regions.
Bootstrap values are shown by nodes.

Figure S5. Coalescent-based species tree from nuclear protein-coding regions. Bootstrap values are shown by nodes.

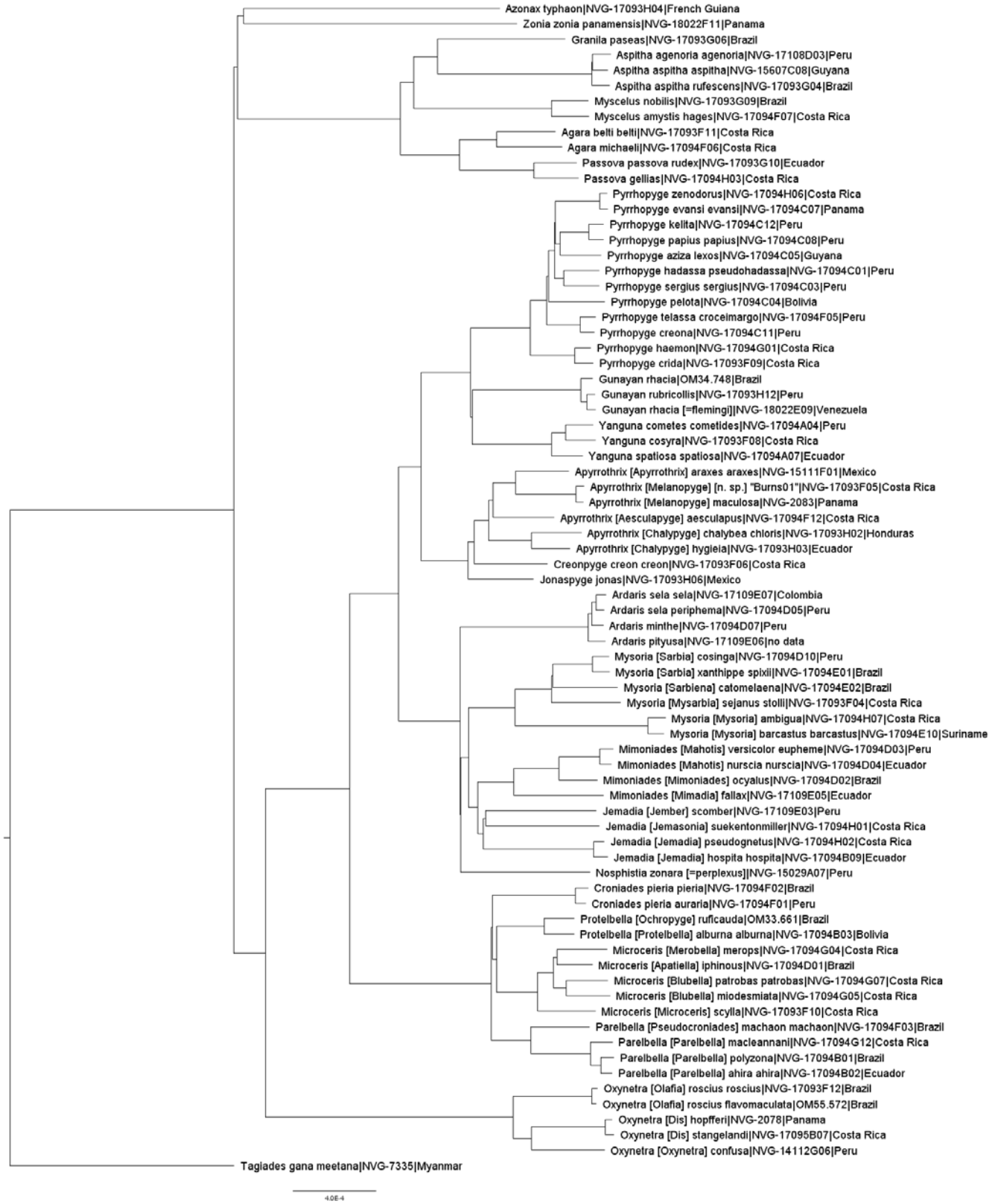

Figure S6. Maximum likelihood tree by TreeMix constructed from nuclear protein-coding regions.

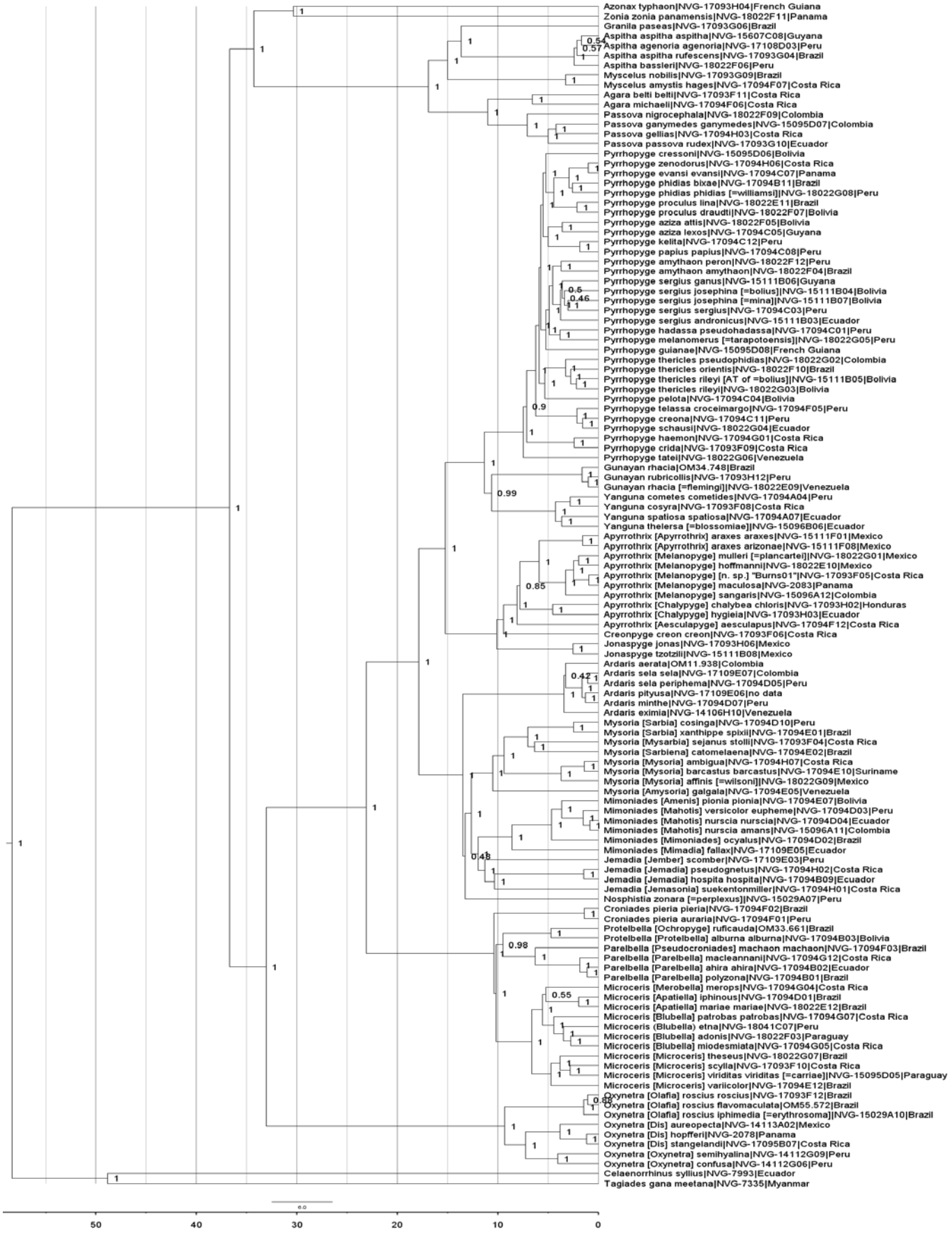

Figure S7. BEAST time-calibrated tree from nuclear protein-coding regions. The posterior probabilities are shown by nodes.

Diagnostic nucleotide characters mapped to the reference genome of *Cecropterus lyciades*

Sequences of nuclear exons with diagnostic characters for the new subtribes listed in the main text are given. The position used as a character state is highlighted in yellow. Base pair in this position is the one present in the *C. lyciades* reference genome, and may not correspond to the ancestral base pair in Pyrrhopyginae. A reference sequence for the COI barcode region is given at the end. Many positions of the barcode are used as diagnostic characters, positions are numbered according to this sequence.

>aly300.8.1:G95T | Amyloid protein-binding protein 2
ATGGCTGACGCGTCGTCGTCGGTGCGCGCAAAGAAAATACCCGATAATCTTTATGAACTGTGTTTGACAAATTTAGT
GAACTATCTGCAGAAATGTAAGTGCGAGCGAAATGATTTGCATTACCTGCCGGACACCGTCCTTATGGATGTGTACT
ACAAG

>aly1838.7.1:T90C | Protein SDA1 homolog
ATGGTGCGGCATAATAATCAGTTACCAAATAACTTGACACAGTTACAAAATCTAATAAAGAGAGATCCAGATTCATA
TAAAGATGAATTTCGACAGCAACTCGCTCACTTTGAAACAACACTTGAGATTTTCAATCTCAACCCTACGCAGTATA
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>aly60.16.9:C66T | Glucose dehydrogenase [FAD, qui]
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ATTTATGCA

>aly2612.6.2:T640C | Serine/threonine-protein kinase SMG1
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>aly2548.11.2:A71G | Function unknown
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>aly822.26.1:A174G | Protein-lysine methyltransferase METTL21D
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>aly7758.8.1:C31A | Protein LLP homolog
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>aly318.14.16:T4044C | Cadherin-related tumor suppressor
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>aly990.1.14:A84G | Down syndrome cell adhesion molecule-like protein Dscam2
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>aly851.9.1:A186G | DNA polymerase epsilon subunit 3
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>aly276558.16.1:T219C | Cyclin-related protein FAM58A
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>aly276558.16.1:T222C | Cyclin-related protein FAM58A
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>aly536.39.1:G60A | 3-phosphoinositide-dependent protein kinase 1
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>aly536.115.1:A576G | YTH domain-containing protein 1
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>aly207.4.1:T58C | Anaphase-promoting complex subunit 4
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>aly2954.5.2:C185G | Integrator complex subunit 3 homolog
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