

**SI Appendix to "Genomes reveal drastic and recurrent phenotypic divergence in  
Firetip skipper butterflies (Hesperiidae: Pyrrhopyginae)" by Jing Zhang, Qian  
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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 & Boulet, 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), *Sarbienna* Grishin, **subgen. n.** (TS: *Sarbia catomelaena* Mabille & Boulet, 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 & Boulet, 1908), *Mysoria pertyi* (Plötz, 1879), *Mysoria curitiba* (O. Mielke & Casagrande, 2002), *Mysoria soza* (Evans, 1951), *Mysoria oneka* (Hewitson, 1866), *Mysoria catomelaena* (Mabille & Boulet, 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 & Boulet, 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 Table 1 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** Mabilite, 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: *zonias* Evans, 1951

#### Tribe **Passovini** Mielke, 2001

Genus **Granila** Mabilite, 1903; TS: *paseas* Hewitson, 1857

Genus **Aspitha** Evans, 1951; *aspitha* Hewitson, [1866]

Genus **Myscelus** Hübner, [1819]; TS: *nobilis* Cramer, [1777]

Genus **Agara** Mabilite & Boulet, 1908; reinstated status; TS: *pardalina* C. Felder & R. Felder, 1867

Genus **Passova** Evans, 1951; TS: *passova* Hewitson, [1866]

#### Tribe **Pyrrhopygini** Mabilite, 1877

Subtribe **Pyrrhopygina** Mabilite, 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 *Chalpyge* 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* Mabilite, 1903; new synonym; TS: *cosinga* Hewitson, 1874

Subgenus *Sarbiena* Grishin, new subgenus; TS: *catomelaena* Mabilite & Boulet, 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* Mabilles, 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* Mabilles, 1878  
 Genus *Nosphistia* Mabilles & Boulet, 1908; TS: =*perplexus* Mabilles, 1878 (*zonara* Hewitson, [1866])

#### Subtribe *Microcerisina* Grishin, **new subtribe**

- Genus *Croniades* Mabilles, 1903; TS: *peria* Hewitson, 1857  
 Genus *Protelbella* O. Mielke, 1995; TS: *alburna* Mabilles, 1891  
 Subgenus *Ochropyge* O. Mielke, 2002; **new status**; TS: *ruficauda* Hayward, 1932  
 Subgenus *Protelbella* O. Mielke, 1995; TS: *alburna* Mabilles, 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* Ménétrés, 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* Mabilles & Boulet, 1908  
 Subgenus *Microceris* E. Watson, 1893; TS: *variicolor* Ménétrés, 1855  
 =*Elbella* Evans, 1951; **new synonym**; TS: *scylla* Ménétrés, 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* Mabilles, 1889; **new status**; TS: =*annulatus* Mabilles 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: *zonía* 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 & Boulet, 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: *aesculapys* 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 & Boulet, 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 & Boulet, 1908; TS: =*perplexus* Mabille, 1878 (*zonara* Hewitson, [1866])

Genus **Microceris** E. Watson, 1893; TS: *variicolor* Ménétriés, 1855

Subgenus *Croniades* Mabille, 1903; TS: *peria* Hewitson, 1857

Subgenus *Ochropyge* O. Mielke, 2002; TS: *ruficauda* Hayward, 1932  
Subgenus *Protellabella* O. Mielke, 1995; TS: *alburna* Mabille, 1891  
Subgenus *Pseudocroniades* O. Mielke, 1995; TS: *machaon* Westwood, 1852  
Subgenus *Parellabella* 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 & Boulet, 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: *zonía* 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 & Boulet, 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 & Boulet, 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 & Boulet, 1908; TS: =*perplexus* Mabille, 1878 (*zonara* Hewitson, [1866])



Subtribe **Microcerisina** Grishin, new subtribe

- Genus **Croniades** Mabille, 1903; TS: *peria* Hewitson, 1857  
Genus **Ochropyge** O. Mielke, 2002; TS: *ruficauda* Hayward, 1932  
Genus **Protellabella** O. Mielke, 1995; TS: *alburna* Mabille, 1891  
Genus **Pseudocroniades** O. Mielke, 1995; TS: *machaon* Westwood, 1852  
Genus **Parellabella** 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: *iphius* Latreille, [1824]  
Genus **Hegesippe** Evans, 1951; TS: *hegesippe* Mabille & Boulet, 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 Table 1 of the main text

Page limits on the main text forced us to keep diagnoses minimal. In Table 1 of 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](https://zoobank.org/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](https://zoobank.org/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 a sharp point, discocellular vein on hindwing concave towards outer margin, veins CuA<sub>1</sub> & M<sub>3</sub> and M<sub>1</sub> & RS wide apart at their origins, end of abdomen brown (if red-orange, then wings unspotted and hindwing crenulate at the 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], *Chalpyge* 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](https://zoobank.org/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<sub>1</sub> & M<sub>3</sub> close to each other at their origins and forewing vein M<sub>3</sub> in the middle between veins M<sub>2</sub> and CuA<sub>1</sub> at their origins (not between veins M<sub>1</sub> and CuA<sub>1</sub>).

**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], *Sarbienna* 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* Mabilite & Boulet, 1908.

**Parent Taxon:** Tribe Pyrrhopygini Mabilite, 1877.

#### **Subtribe *Microcerisina* Grishin, subtr. n.**

**Type genus:** *Microceris* E. Watson, 1893. **ZooBank registration:** [2D1DB769-9A47-4BD1-A66D-9CD937825113](https://zoobank.org/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* Mabilite, 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 Mabilite, 1877.

#### **Subgenus *Aesculapyge* Grishin, subgen. n.**

**Type species:** *Pyrrhopyge aesculapus* Staudinger, 1876. **ZooBank regist.:** [D6952953-3744-402D-9A01-9D88246DAB47](https://zoobank.org/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 its base.

**Species included:** *Pyrrhopyge aesculapus* Staudinger, 1876.

**Parent Taxon:** Genus *Apyrrothrix* Lindsey, 1921.

#### **Subgenus *Sarbiena* Grishin, subgen. n.**

**Type species:** *Sarbia catomelaena* Mabilite & Boulet, 1908. **ZooBank reg.:** [D76F2A12-DB82-46E3-A06E-3EA038A0B0E0](https://zoobank.org/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<sub>1</sub>, palpi terminally orange-red. Uncus broad, lacks dorsally directed spike, arms long, distant from each other.

**Species included:** *Sarbia catomelaena* Mabilite & Boulet, 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](https://zoobank.org/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](https://zoobank.org/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](https://zoobank.org/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](https://zoobank.org/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](https://zoobank.org/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](https://zoobank.org/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](https://zoobank.org/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](https://zoobank.org/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 Tab. 1). 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 Table 1). 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 Table 1). 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 Table 1).

*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, Table 1 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 were 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* Mabilbe & Boulet, 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 Table 1.

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 Table 1.

### 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 |
- Incubate at 20°C for 15 minutes in a thermal cycler.

#### D. USER excision

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

#### E. Cleanup of Adaptor-ligated DNA

- Move AMPure XP Beads to room temperature for 20 min. Vortex beads to resuspend.
- 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.
- Incubate for 10 minutes at room temperature.
- 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.
- 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.
- Repeat Step 5 once.
- Air dry the beads.
- Remove the tube/plate from the magnet. Elute the beads twice with 17 µl TE buffer.
- Mix well by pipetting up and down. Incubate for 5 minutes at 37°C.
- Quickly spin the tube and place it on the magnetic stand.
- After the solution is clear (about 5 minutes), transfer 15 µl of the elution to a new PCR well plate.
- Elute a 2nd time with 10 µl, to a final volume of 25 µl.
- Measure concentration using Promega QuantiFluor® dsDNA System with 1 µl.

#### F. PCR Enrichment of Adaptor Ligated DNA

- 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
- 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.

<i>CYCLE STEP</i>	<i>TEMP</i>	<i>TIME</i>	<i>CYCLES</i>
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  $\mu$ l.
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  $\mu$ l 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  $\mu$ l of 10 mM Tris-HCl 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  $\mu$ l to a new well plate.
13. Elute a 2nd time with 19  $\mu$ l, to a final volume of 37  $\mu$ l.
14. Measure libraries concentration with 2  $\mu$ l.

### *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  $\geq 95\%$ ) 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  $\log(e\text{-value}) < 5$ ), 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  $\geq 75\%$ , (b) identity to the query  $\geq 50\%$ , and  $\geq$  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.

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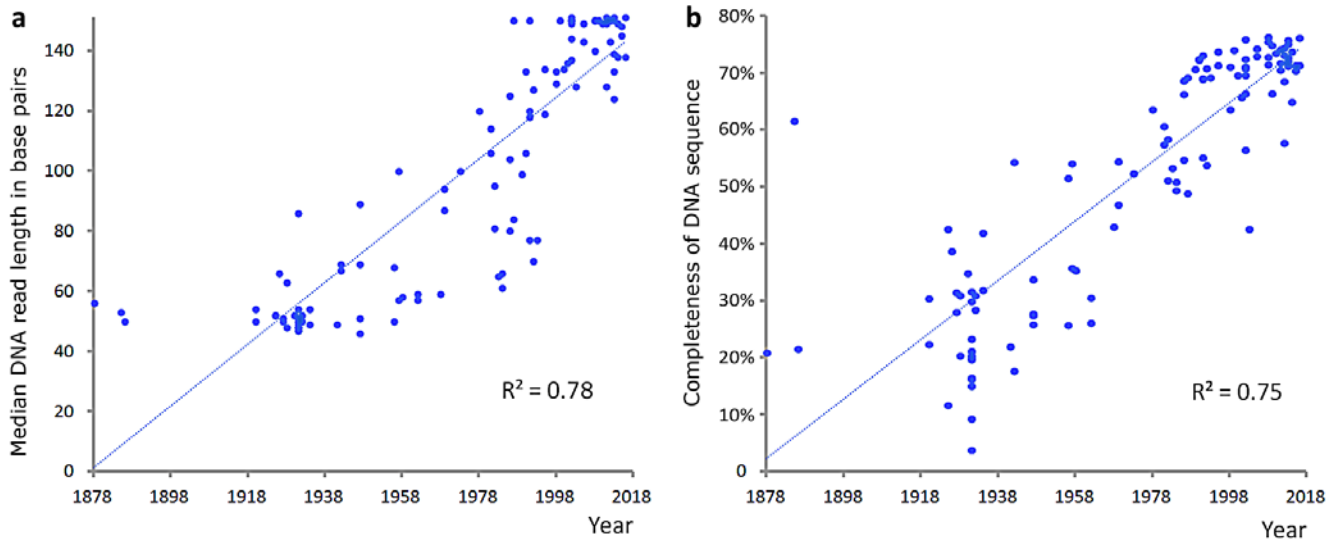
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Table S1. Data for 119 sequenced Pyrrhopyginae specimens and 2 outgroups

#	DNA Voucher	Taxon name	Sex	Locality	Elevation	GPS	Collectors	Date	Collection	Genitalia No.	Collection No.
1	1 NVG-17093H04	<i>Azonox typhaon</i>	HT	M French Guiana: nr. Cayenne, Montagne aux chevaux (Gallon)		4.7250, -52.4083	Jean-Yves Gallard	7-Apr-1991	USNM		
2	2 NVG-18022F11	<i>Zonia zonia panamensis</i>	HT	Panamá: Canal Zone, Madden Forest Preserve			Stanton S. Nicolay	6-Feb-1968	AMNH	H379	
3	3 NVG-17093G06	<i>Gramila pasceus</i>	HT	Brazil: Rio de Janeiro, Tijuca	165 m	-10.53, -62.80	Collection W. Schauss	N/A (old)	USNM		USNMMENT 00894724
4	4 NVG-17093G04	<i>Asapha asapha rufescens</i>	F	Brazil: Rondônia, 62 km S Ariguanes, Fazenda Rancho Grande	150-300 m	3.3450, -59.5700	Ron Leuschner	29-Sep-10-Oct-1992	USNM		USNMMENT 00233032
5	5 11-80-A-15607C08	<i>Asapha asapha asapha</i>	F	Guyana: Upper Takutu-Upper Essequibo (Región 9), Kamuku Mts., Nappu Creek	550 m		S. Fraiello, K. Hamms, S. Hendricks, R. Williams	21-Feb-10-Apr-1999	USNM	G1408	
6	6 NVG-18022P06	<i>Asapha bossieri</i>	HT	M Peru: San Martín, Achimanziza				11-Sep-1927	AMNH		
7	7 NVG-17108D03	<i>Asapha agenorita agenorita</i>	M	Peru: Huánuco Region, 15 mi S Tringo María, Chinchavito				24-Jun-1982	LACM		
8	8 NVG-17093G09	<i>Myoscielus amyntis hages</i>	M	Brazil: Rondônia, 8 km N Caculândia	290 m	-10.50, -62.87	D. H. Ahrenholz	5-Nov-1989	USNM		USNMMENT 00894703
9	9 NVG-17094F07	<i>Myoscielus amyntis hages</i>	M	Costa Rica: Guanacaste Prov., ACG, Sector Del Oro, Quebrada Suamposa	980 m	11.02364, -85.49139	Lucia Rios	eclosed on 13-Mar-2005	USNM		05-SRNP-20512
10	10 NVG-17094F11	<i>Agara belli belli</i>	M	Costa Rica: Alajuela Prov., ACG, Sector Rincon Rain Forest, Sendero Albergue Crater	670 m	10.84886, -85.32810	Carolina Cano	eclosed on 19-Sep-2009	USNM		09-SRNP-4464
11	11 NVG-17094F06	<i>Agara michaeli</i>	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	G419	USNMMENT 00894700
12	12 NVG-18022F09	<i>Passova nigroccephala</i>	HT	M Colombia				prior to 1934	AMNH		
13	13 NVG-15095D07	<i>Passova nigroccephala</i>	HT	M Colombia				1925	CMNH	Slide No. 1957	
14	14 NVG-17094H03	<i>Passova geyllies</i>	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	15 NVG-17093G10	<i>Passova passova nudex</i>	M	Ecuador: Napo Prov., 4 km Tena-Pano Rd.	600 m	-1.03, -77.83	Stanton S. Nicolay	28-Sep-1990	USNM		USNMMENT 00894708
16	16 NVG-15095D06	<i>Pyrrhopyge cressoni</i>	HT	M Bolivia: Santa Cruz, 75 km NW of Buenavista	450 m		Jose Steinbach	1926-1927	CMNH	Slide No. 1978	
17	17 NVG-17094H06	<i>Pyrrhopyge evanorisi</i>	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	18 NVG-17094H07	<i>Pyrrhopyge evanorisi</i>	M	Panamá: Veraguas, Isla Coliba, Represa		7.50, -81.70	G. B. Small	25-Feb-1981	USNM	V38 S. S. Nicolay	USNMMENT 00894891
19	19 NVG-17094B11	<i>Pyrrhopyge phidias phidias (=williamsi)</i>	HT	M Peru				Nov-1962	USNM		USNMMENT 00894883
20	20 NVG-18022G08	<i>Pyrrhopyge phidias phidias (=williamsi)</i>	HT	M Brazil: Pará			A. M. Moss	prior to 1931	AMNH	G423	
21	21 NVG-18022E07	<i>Pyrrhopyge procallus lina</i>	HT	M Bolivia: Santa Cruz				prior to 1947	AMNH	G1812	
22	22 NVG-18022E07	<i>Pyrrhopyge procallus draudti</i>	HT	M Bolivia: Santa Cruz				prior to 1931	AMNH		
23	23 NVG-17094C05	<i>Pyrrhopyge aziza texas</i>	M	Guyana: Iwokrama Mts, Iwokrama Rainforest Reserve	750-950 m	4.3303, -58.7985	S. Fratello	28-Mar-1-Apr-2001	USNM	G451	USNMMENT 00179063
24	24 NVG-18022F05	<i>Pyrrhopyge aziza texis</i>	HT	M Bolivia: Santa Cruz			A. M. Moss	prior to 1947	AMNH	G1813	
25	25 NVG-18022F10	<i>Pyrrhopyge therides onemits</i>	HT	M Brazil: Pará				prior to 1931	AMNH	G433	
26	26 NVG-18022G02	<i>Pyrrhopyge therides pseudophidias</i>	HT	M Bolivia: Muzo	400 m			prior to 1931	AMNH	G433	
27	27 NVG-18022G03	<i>Pyrrhopyge therides rileyi</i>	HT	M Bolivia: Santa Cruz				prior to 1931	AMNH		
28	28 NVG-15111B05	<i>Pyrrhopyge therides rileyi (AT of =bolius)</i>	F	Bolivia: Dept. Santa Cruz, Buena Vista				prior to 1931	AMNH		
29	29 NVG-18022F12	<i>Pyrrhopyge amythaon peron</i>	HT	M Peru: Iquitos				prior to 1947	AMNH	G687	
30	30 NVG-18022F04	<i>Pyrrhopyge amythaon amythaon</i>	HT	M Brazil: Pernambuco				16-Feb-1932	AMNH	G1179	
31	31 NVG-15111B06	<i>Pyrrhopyge sergius ganus</i>	HT	M Guyana			C. L. Pollard	prior to 1947	AMNH	G1262	
32	32 NVG-15111B04	<i>Pyrrhopyge sergius josephina (=bolius)</i>	HT	M Bolivia: R. Chimato	580 m			12-Apr-1926	AMNH	G1720	
33	33 NVG-15111B07	<i>Pyrrhopyge sergius josephina (=bolius)</i>	HT	M Bolivia				prior to 1931	AMNH	G416	
34	34 NVG-15111B03	<i>Pyrrhopyge sergius andronicus</i>	HT	M Ecuador: Macas				prior to 1931	AMNH	G383	
35	35 NVG-17094C03	<i>Pyrrhopyge sergius sergius</i>	HT	M Peru: Cuzco, Cosnipata Rd., San Pedro Lodge	1450 m		Steve Kinyon	6-Dec-2012	USNM		USNMMENT 00894898
36	36 NVG-15095D08	<i>Pyrrhopyge guinnee</i>	HT	M French Guiana				before 1932	CMNH		
37	37 NVG-17094C04	<i>Pyrrhopyge pelata</i>	M	Bolivia: Beni, 40 km E San Borja Estacion Biologica Beni, Palm Camp	1700 m		M. G. Pogue	11-Sep-1987	USNM		USNMMENT 00894879
38	38 NVG-17094C12	<i>Pyrrhopyge kelta</i>	M	Peru: Cuzco, Mirador, Cosnipata Rd.	1800 m		Steve Kinyon	5-Nov-2012	USNM		USNMMENT 00894866
39	39 NVG-17094C08	<i>Pyrrhopyge papus papus</i>	M	Peru: San Martín, Rio Serranoyacu				11-Nov-1998	USNM		USNMMENT 00894901
40	40 NVG-18022G05	<i>Pyrrhopyge melanomerus (=tarapotoensis)</i>	HT	M Peru: Tarapoto				prior to 1931	AMNH		
41	41 NVG-17094C01	<i>Pyrrhopyge hadassa pseudohadassa</i>	HT	M Peru: Cuzco, Cosnipata Valley	1375 m	-5.667, -77.750	Robert K. Robbins & Gerardo Lamas	11-Oct-2014	USNM	G424	USNMMENT 00894877
42	42 NVG-17094C11	<i>Pyrrhopyge creona</i>	M	Peru: Amazonas, Abra Pardo Miguel	2200 m	-5.70, -77.80	Steve Kinyon	11-Jun-2011	USNM		USNMMENT 00894876
43	43 NVG-17094C11	<i>Pyrrhopyge creona</i>	M	Peru: Cuzco, Cosnipata Rd., San Pedro Lodge	1450 m		Steve Kinyon	prior to 1931	AMNH		USNMMENT 00894765
44	44 NVG-18022G04	<i>Pyrrhopyge telassa croceimargo</i>	HT	M Ecuador				prior to 1931	AMNH		
45	45 NVG-17094G01	<i>Pyrrhopyge haemon</i>	F	Costa Rica: ACG	525 m	11.01373, -85.42531	Roster Moraga	2002	USNM		02-SRNP-35242
46	46 NVG-17093F09	<i>Pyrrhopyge crida</i>	HT	M Costa Rica: Guanacaste Prov., ACG, Sector Del Oro, Monte Cristo				eclosed on 04-Apr-2009	USNM		09-SRNP-20790
47	47 NVG-18022G06	<i>Pyrrhopyge tatei</i>	HT	M Venezuela: Provisional Camp, Mt. Duida				12-Dec-1928	AMNH	G1177	
48	48 OM34.748	<i>Guayana rhacia</i>	M	Brazil: Santa Catarina, Joinville			O. H. Mielke	15-Feb-1981	OM-DZUP		
49	49 NVG-17093H12	<i>Guayana rubricollis</i>	HT	M Venezuela: Caripito			D. H. Ahrenholz	5-Oct-1986	USNM		USNMMENT 00894904
50	50 NVG-18022E10	<i>Guayana rhacia (=flemingi)</i>	HT	M Venezuela: Caripito			Fleming	29-Jul-1942	AMNH	G1931	
51	51 NVG-17094A04	<i>Yanguana telassa (=blossomiare)</i>	HT	M Ecuador: Huigra	300 m	-12.83, -69.28	W. J. Coxey	6-Oct-1986	CMNH		USNMMENT 00894884
52	52 NVG-15096B06	<i>Yanguana telassa (=blossomiare)</i>	HT	M Ecuador: Huigra	1200 m			May-1930	CMNH		
53	53 NVG-17093F08	<i>Yanguana spatiosa spatiosa</i>	M	Costa Rica: Guanacaste Prov., ACG, Sector Pitilla, Sendero Oroslito	900 m	10.98332, -85.43623	Manuel Pereira	eclosed on 01-Oct-2008	USNM		08-SRNP-31712
54	54 NVG-17094A07	<i>Yanguana spatiosa spatiosa</i>	M	Ecuador: Zamora-Chinchipe, 24 km Zamora-Loja Rd., Quebrada San Ramon	1700 m		David H. Ahrenholz	15-Jan-2002	USNM		USNMMENT 00894887
55	55 NVG-15111F08	<i>Apryrrhox (Apryrrhox) araxes arizanae</i>	HT	M Mexico: Sonora, approx. 18 mi W. Camaneta	2600 m	-3.9693, -79.0625	Peter Hubbell	26-Aug-1969	AMNH		
56	56 NVG-15111F01	<i>Apryrrhox (Apryrrhox) araxes araxes</i>	HT	M Mexico: Hidalgo, Cuesta Colorado			William H. Howe	2-Sep-1978	AMNH		
57	57 NVG-18022G01	<i>Apryrrhox (Melaanopyge) maliari (=pliancarte)</i>	HT	M Mexico: Veracruz, Presidio				prior to 1941	AMNH		
58	58 NVG-18022G01	<i>Apryrrhox (Melaanopyge) maliari (=pliancarte)</i>	HT	M Mexico: Veracruz, Presidio				prior to 1941	AMNH		
59	59 NVG-17093F05	<i>Apryrrhox (Melaanopyge) maliari (=pliancarte)</i>	HT	M Mexico: Tabasco, Tenosique	730 m	10.86739, -85.38744	E. C. Welling	3-Sep-1962	AMNH		14-SRNP-2944
60	60 NVG-2083	<i>Apryrrhox (Melaanopyge) maliari (=pliancarte)</i>	HT	M Panama: Panama, Gambia, Pipeline road	75 m	9.12717, -79.17483	John R. MacDonald	eclosed on 03-Sep-2014	USNM		
61	61 NVG-15096A12	<i>Apryrrhox (Melaanopyge) maliari (=pliancarte)</i>	HT	M Colombia: Magdalena, Sierra San Lorenzo, Hacienda Cincinnati	1400 m		Rehn and Hebard	2-Jun-2012	MEV		
62	62 NVG-17093H02	<i>Apryrrhox (Melaanopyge) maliari (=pliancarte)</i>	HT	M Ecuador: Zamorano				12-Aug-1957	USNM		USNMMENT 00894713
63	63 NVG-17093H03	<i>Apryrrhox (Chalyptopge) chalyptopge</i>	HT	M Ecuador: Esmeraldas, Rio Chuchivi, km 12.5 Lita-San Lorenzo Rd.	800-900 m	0.8835, -78.5150	I. Aidas	1-May-2002	USNM		USNMMENT 00894709
64	64 NVG-17094F12	<i>Apryrrhox (Chalyptopge) hygieia</i>	F	Costa Rica: Guanacaste Prov., ACG, Sector Cacao, Sendero Cima	1460 m	10.93328, -85.45729	Manuel Pereira	eclosed on 18-Jun-2011	USNM		10-SRNP-35667
65	65 NVG-17093F06	<i>Creonopyge (Aesclapogae) aesculapug</i>	M	Costa Rica: Guanacaste Prov., ACG, Sector Santa Maria, Santa Maria	840 m	10.75772, -85.30598	Calisto Moraga	eclosed on 13-Jan-2015	USNM		15-SRNP-30170
66	66 NVG-17093H06	<i>Creonopyge creon creon</i>	M	Mexico: Oaxaca, Sierra Juarez, Gulf slope	1800-2100 m		John Kemner	2-Aug-1991	USNM		USNMMENT 00894867
67	67 NVG-15111B08	<i>Jonessyge jonas</i>	HT	M Mexico: Chiapas, Ocozingo			T. Escalante	Jul-1942	AMNH		
68	68 OM11.938	<i>Atadaris eximia</i>	F	Colombia: Sierra Nevada de Santa Marta			A. Schultze	19-Mar-1928	OM-DZUP		
69	69 NVG-14106H10	<i>Atadaris eximia</i>	F	Venezuela: Merida, Paramo La Culata	3200-3400 m		C. Bordon	6-Aug-1984	USNM		
70	70 NVG-17109E07	<i>Atadaris seta seta</i>	M	Colombia: Prov. Santa Maria, Boyaca	1373 m	-13.0583, -71.5344	Brian Harris	10-Mar-1983	LACM		USNMMENT 00894770
71	71 NVG-17094D05	<i>Atadaris seta peripherna</i>	M	Peru: Cuzco, Cosnipata Valley, San Pedro vicinity	950 m			6-Feb-2011	LACM		USNMMENT 00894773
72	72 NVG-17109E06	<i>Atadaris ptyusa</i>	M	no data	3150 m			N/A	LACM		USNMMENT 00894776
73	73 NVG-17094D07	<i>Atadaris ptyusa</i>	M	Peru: San Martín, Puente Aguas Verdes	800 m			10-Nov-1998	USNM		USNMMENT 00894773
74	74 NVG-17094D10	<i>Mysoiria (Sarbia) selanippe spixii</i>	F	Peru: Cuzco, Quebrada Chusa, km 88, Paucartambo/Agjancas Rd.	680 m	10.98738, -85.41967	Petrona Rios	21-Jan-1973	USNM		USNMMENT 00894776
75	75 NVG-17094F01	<i>Mysoiria (Mysoiria) selanippe spixii</i>	F	Brazil: Santa Catarina, Sao Bento do Sul, Rio Vermelho	1250 m	-19.267, -48.583	Robert K. Robbins & Becker	11-Feb-2010	USNM		10-SRNP-30468
76	76 NVG-17093F04	<i>Mysoiria (Sarbia) selanippe spixii</i>	F	Costa Rica: Guanacaste Prov., ACG, Sector Pitilla, Sendero Misimo	330 m	10.76261, -85.42979	Jose Cortez	17-Apr-1991	USNM		USNMMENT 00894777
77	77 NVG-17094E07	<i>Mysoiria (Sarbia) selanippe spixii</i>	F	Brazil: Mato Grosso, Serra do Cipo				22-Oct-2013	USNM		13-SRNP-65544
78	78 NVG-17094H07	<i>Mysoiria (Mysoiria) barcastus barcastus</i>	HT	M Suriname: Commewijne De Nieuwe Grond	300 m		Olle Pellmyr	6-Feb-1982	USNM		USNMMENT 00894755
79	79 NVG-17094E10	<i>Mysoiria (Mysoiria) affinis (=wilsoni)</i>	M	Mexico: Guerrero, Mexcala			Kent Wilson	22-Jul-1956	AMNH		USNMMENT 00894750
80	80 NVG-18022G09	<i>Mysoiria (Mysoiria) affinis (=wilsoni)</i>	M	Venezuela: Coniedes, San Carlos Bridge			D. H. Ahrenholz	8-Sep-2003	USNM		USNMMENT 00894752
81	81 NVG-17094E05	<i>Mimonoides (Amenis) planitia planitia</i>	M	Bolivia: Chuguisaca Prov., 30 km SE Carandady	1375 m		Stephen C. Bromley	20-Oct-2013	USNM		USNMMENT 00894881
82	82 NVG-17094E07	<i>Mimonoides (Mahotis) versicolor eupheme</i>	M	Peru: Cuzco, Cosnipata Valley, San Pedro Lodge	1600 m	-2.2617, -78.2033	I. Aidas	16-Oct-2002	USNM		USNMMENT 00894855
83	83 NVG-17094D03	<i>Mimonoides (Mahotis) versicolor eupheme</i>	M	Ecuador: Morona-Santiago, Rio Abamico					USNM		
84	84 NVG-17094D04	<i>Mimonoides (Mahotis) versicolor eupheme</i>	M	Ecuador: Morona-Santiago, Rio Abamico					USNM		



## 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 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.

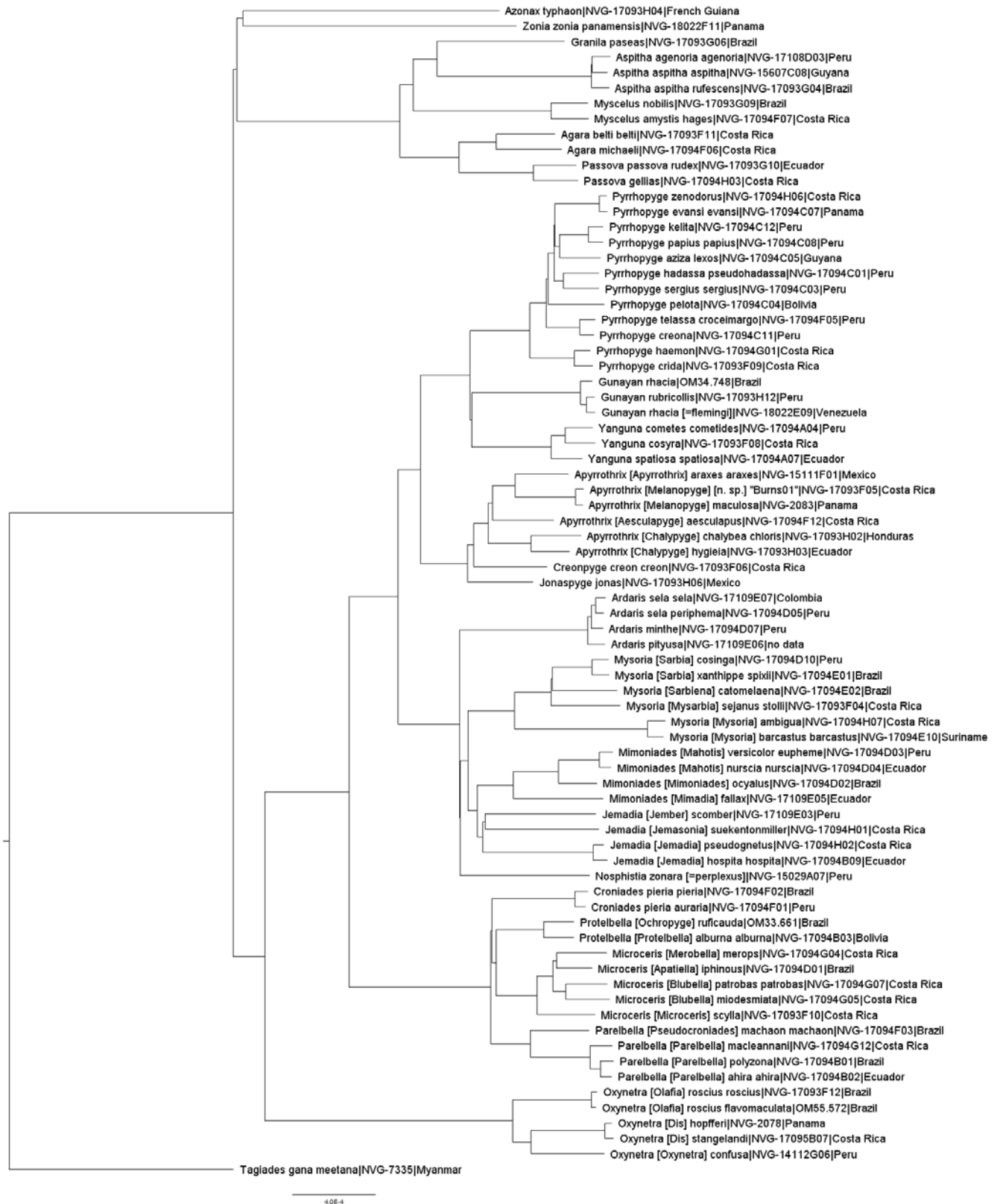


Figure S6. Maximum likelihood tree by TreeMix constructed from nuclear protein-coding regions.

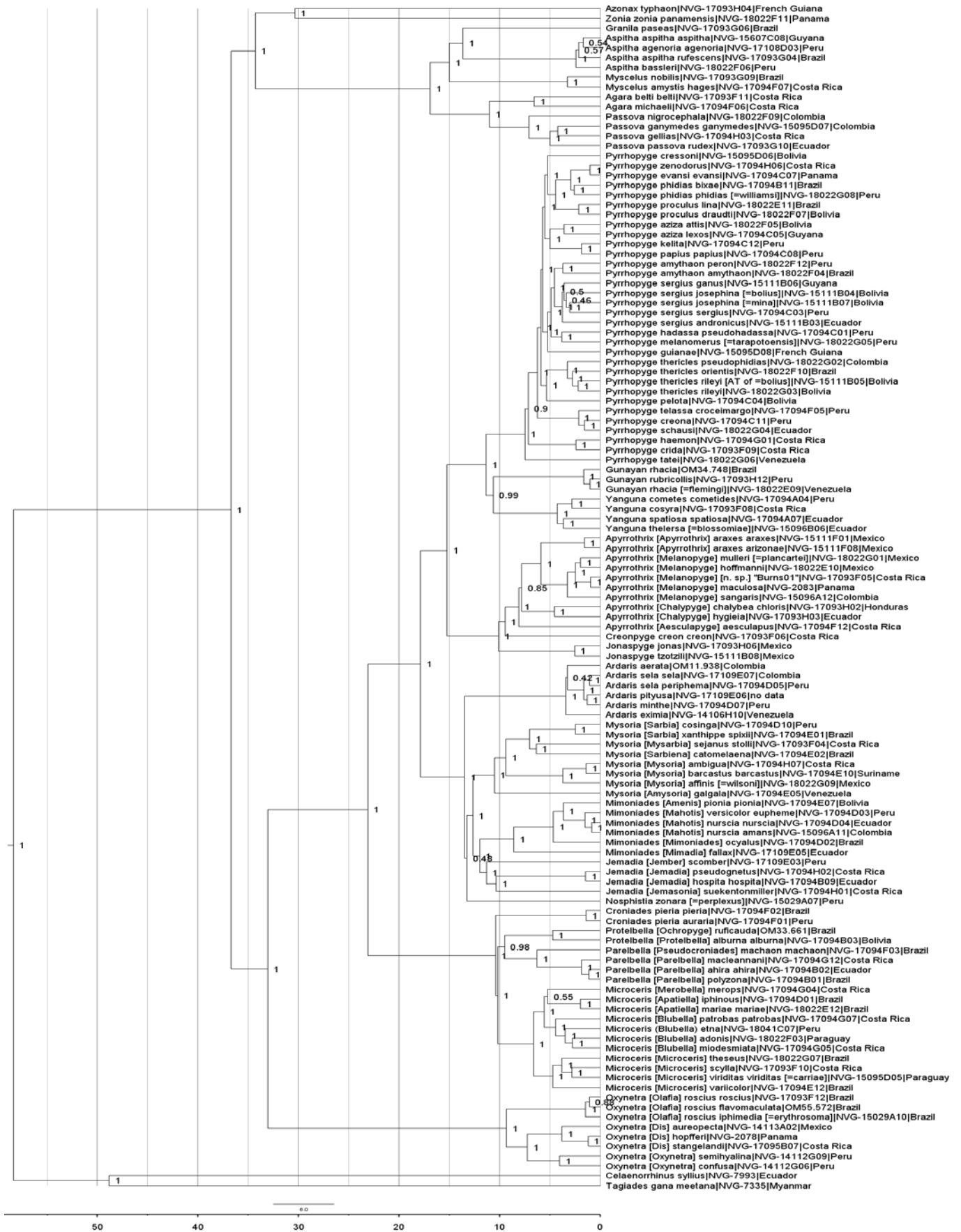


Figure S7. BEAST time-calibrated tree from nuclear protein-coding regions. The posterior probabilities are shown by nodes.

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

Sequences of nuclear exons with diagnostic characters for the new subtribes listed in Table 1 (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 characters in Table 1, positions are numbered according to this sequence.

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

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ACAAG
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>aly1838.7.1:T90C | Protein SDA1 homolog

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>aly60.16.9:C66T | Glucose dehydrogenase [FAD, qui]

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>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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>COI barcode reference sequence, many positions are used as characters

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