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Calopteryx splendens and relatives: taxonomy, biogeography, and phylogeny (Odonata: Calopterygidae)

Henri J. Dumont[1], Sergey Borisov[2], Thomas Schneider[3],
Dietmar Ikemeyer[4], Michel Papazian[5],
Matti Hämäläinen[6] & Andy Vierstraete[1]

[1] Department of Biology, University of Gent, Ghent, Belgium;
corresponding author: Henri.Dumont@UGent.be
[2] Institute of Systematics and Ecology of Animals, Russian Academy of Sciences,
Siberian Branch, Frunze str. 11, Novosibirsk 630091, Russia;
borisov-s-n@yandex.ru
[3] Arnold-Knoblauch-Ring 76, 14109 Berlin-Wannsee, Germany;
thomas.rs@gmx.de
[4] Billerbecker Straße 6, 48329 Havixbeck, Germany; DKJIkemeyer@t-online.de
[5] Muséum d'Histoire naturelle de Marseille, Palais Longchamp, France;
papazianmichel@orange.fr
[6] Naturalis Biodiversity Center, Leiden, The Netherlands;
libellago@gmail.com

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Abstract. We regard species, subspecies, and genera as measurable steps in an evolutionary process that requires time. Many species seem to require about one million years to become sufficiently distinct to be considered distinct. We test this in the damselfly genus *Calopteryx* Leach in Brewster, 1815, and especially in the group of *Calopteryx splendens,* trying to reconcile morphology with biogeography, including glaciations, and molecular phylogeny. We find that the *C. splendens*-group consists of four species, supported by distribution, morphology (wing patch size), COI, and ITS 1 and 2 (fragments of DNA), viz. *C. exul* Selys, 1853, *C. samarcandica* Bartenev, 1912, *C. splendens* (Harris, 1780), and *C. xanthostoma* (Charpentier, 1825). Only the first one fulfils the requirement of one million years in isolation, but all four seem to resist introgression. Despite enormous interpopulation variation, only two of the *ca* 25 named subspecies of *C. splendens* are considered valid: *Calopteryx s. orientalis* Selys, 1887, and *C. s. syriaca* Rambur, 1842. They began developing distinctive phenotypes during the Würm III deglaciation, *ca* 20 000 years ago, and have not yet reached species status. The taxonomic decisions made in this study include the placing of 17 named subspecies in synonymy with *C. splendens splendens*.

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The last glacial maximum without doubt saw dramatic reductions in dragonfly populations that survived only in refugia. The Mediterranean basin was one of these, and central Asia another, more complex one. The western part of the basin, mainly Iberia, is home to *C. xanthostoma*, a species that recently became extinct in the Maghreb and is currently being outcompeted from south-western France to Liguria. There it meets *C. splendens* of various wing-patch morphs, coming from central Asia (Irano-Turania), via Anatolia, the Balkans, and Italy. To understand this, we assume two invasion waves occurred with different temperature preferences, causing them to disperse separately. The first one was cold-adapted, without a dark wing patch, and evolved in early preglacial times. It is still found along part of the southern margin of the Black Sea and probably evolved there. The second wave was warm-adapted with wide wing bands and androchrome females. It probably originated near the Zagros Mountains. All extant morphs can be explained as recent mixtures forming continuous clines of the two extremes.

Throughout the Holocene, the Zagros and the Black Sea populations recolonised central Asia and Siberia, eventually reuniting with those in the Baltic zone in the west, but this enormous territory needs much more study. In the south-east, *Calopteryx* did not cross the Pamir Mountain Knot. *Calopteryx* species that have been claimed to occur in the East Palaearctic mainly belong in Oriental genera such as *Atrocalopteryx* Dumont *et al.* 2005. The last common ancestor of American and Eurasian *Calopteryx* is believed to have lived in the Miocene age and migrated between continents via Beringia. It requires a genus name.

Further key words. Dragonfly, damselfly, Zygoptera, speciation, synonyms, *C. exul*, *C. samarcandica*, *C. xanthostoma*, *C. s. orientalis*, *C. s. syriaca*, Eurasia, West Palaearctic

Introduction

Calopterygidae is a family of medium- to large, metallic-bodied Zygoptera of black, blue, green, or bronze to red coloration and with tropical to temperate zone distributions. Adult calopterygids mostly live by running water and are absent from Australia and Antarctica. A total of *ca* 180 species is currently recognized. The highest diversity is reached in Southeast Asia, especially in Indochina and southern China (Hämäläinen & Fliedner 2022). In South America the family is represented by the subfamily Hetaerininae, with high species diversity in the *Hetaerina*/*Mnesarete* group.

The wings may exhibit sexual dimorphism with those of males ranging from hyaline to covered by dark brown-black, red, orange, or iridescent green pigmented bands or patches. Although the wing markings play a role in courtship display and thus in reproductive isolation, their function, especially in androchrome females, is not well understood. In *Calopteryx splendens* and other species of the genus, the wing pigment is melanin, deposited in the cuticle of the wing membrane. The markings are believed to be genetically determined, but the mechanism of inheritance is not understood.

Geographic range

The geographic range of the *Calopteryx splendens*-group is vast and still has not been fully elucidated. Figure 1 is therefore accurate in the south and west but only an approximation in the north, mainly in Siberia. In North Africa, the range extends from the High Atlas of Atlantic Morocco to the Tell Atlas in Tunisia. There are no calopterygids in Libya, Egypt, the Sinai Desert, and Arabia, and they were probably never present there during the Pleistocene. They reappear in the Levant, in Iraq, and Anatolia, and encircle the Black and Caspian Seas but are currently absent from the arid east Caspian Sea basin. They are plentiful in most of Europe including European Russia, except in the subarctic and arctic north. They occur in the southern Urals and are present in wide swathes of Siberia as far east as the middle river Lena and the south end of lake Baikal. In Siberia they extend, roughly, to 55° north, but their northern limit varies from place to place, depending on local climate and topography. In Finland, the range of *C. splendens* reaches the Arctic Circle, whereas *C. virgo* (Linnaeus, 1758) reaches almost 69° north. There are also no *Calopteryx* species in southern and eastern Mongolia, but there are few pioneer populations inside Mongolia, associated with the river Irtysh (Peters 1985; Dumont 2003). The *C. splendens*-group is also absent in Primoriye, far-eastern Siberia, or the Russian Far East. In Central Asia, its eastern limit of occurrence is ragged and determined by a complex cluster of high mountains (up to 8 000 m a.s.l.) that eventually merges with the Himalayas. Especially important are the Pamirs with its subranges, the Tadjik and Kirgiz mountains, Hindu Kush, Karakoram, and Tien Shan – the so-called Pamir Knot. Together, these provide a formidable barrier for dragonflies, hardly surveyed to date. In Central Asia, mainly Kazakhstan, Uzbekistan, and Turkmenistan, where the dominant habitats are semi-deserts and mountain chains, the presence of *Calopteryx* is determined by climate. No *Calopteryx* has been found south and east of the arc formed by the Hindu Kush-Karakoram in the direction of Pakistan. The few calopterygids that have so far been recorded from the south flank of the central Hindu Kush are of Oriental extraction [*e.g.*, *Neurobasis chinensis* (Linnaeus, 1758); TS pers. obs. 2022, NW Pakistan, 34.6465°N, 72.0768°E, 688 m]. The range of *Calopteryx* ends at the Pamir Knot, but the fine details require closer study.

Species richness

In this study, we recognize four species and, including the nominate ssp., three subspecies in the *Calopteryx splendens*-group. In deciding on the status of a taxon, we give equal weight to morphology, *i.e.*, wing shape and size in males and females, wing markings in males, geographical distribution, and molecular phylogeny, *i.e.*, the analysis of DNA information derived from the nucleotide sequence analysis of marker genes. Considering individual variation, we apply the rule of thumb that full species are separated by a minimum 3±1 % of genetic distance (Hebert *et al.* 2003). Applied to *Calopteryx*, this would mean that *C. samarcandica* is of the order of a million years old (in a test of 12 specimens sequenced for COI, the genetic distance varied from 2.4 to 3.1 %, Kimura two-parameter values expressed as percentages; *cf.* Appendix I). This was independently shown by Góral *et al.* (2023), who confirmed species status for *C. samarcandica.* According to Uyeda *et al.* (2011), a million years is also the average lapse of time required

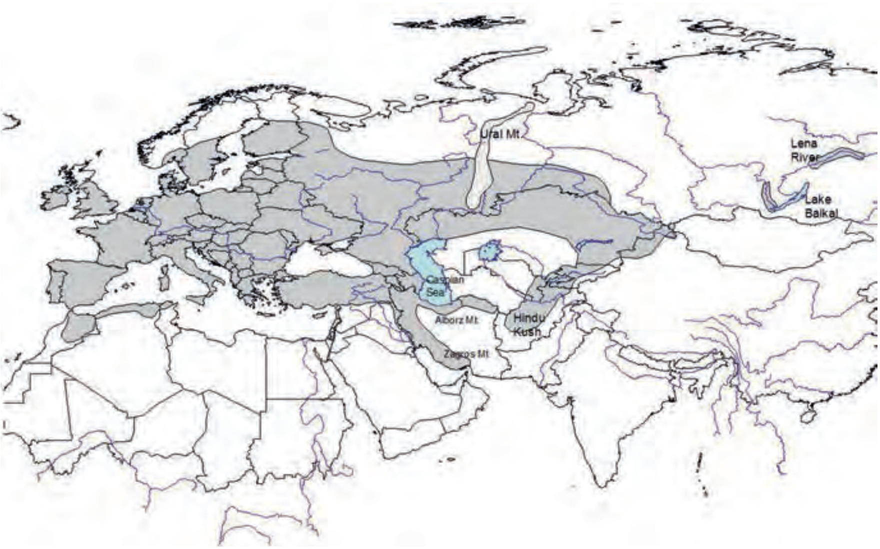

Figure 1. Map of North Eurasia with a rough range of the *Calopteryx splendens*-group. The range covers most of the West Palaearctic, but the northern limits are not exactly known, and the eastern border – currently the middle river Lena system – is also only roughly known.

for speciation, *i.e*., accumulation of mutations, in animals across wide taxonomic boundaries.

Speciation is normally a gradual process, so there must be a point in time, not necessarily halfway, where it becomes irreversible. At that point the number of hybrids produced is insufficient to lead to introgression of the nascent new species and speciation will proceed by reproductive isolation. And although speciation is a genomic process, involving the full complement of genes, different genes, or even parts of genes evolve at different speeds and different genes affect compatibility for cross fertilization. Thus, in our sample of *C. xanthostoma,* and contrary to other examples, COI evolved at a slower pace, while ITS evolved at the usual speed.

Of course, climate also plays a role and evolution benefits from long periods of climatic stability. We therefore accept that past climate changes since the late Miocene set the scene for the present-day faunas.

The influence of climate change, especially glaciation

Around 2.5 million years ago, ice began to accumulate at the North Pole and became permanent on parts of North America, some parts of West–Central Asia, and large parts of West Europe including the British Isles. Ice also covered much of Scandinavia and the coastal zone of European Russia, at times down to the Netherlands. The phenomenon was complex and variable in timing and intensity. Periods during which temperature decreased by 4–6°C or more were followed by warm intervals such as the post-Würmian period or the Anthropocene, in which we currently live.

In all, there were *ca* 20 glaciation events and interglacial periods of variable intensity in Eurasia and America. Life on glaciated territories of Scandinavia, England, Germany, Poland, and the Atlantic zone of Russia went extinct or into survival mode. It was highly selective on tundra and absent on ice-covered grounds. One way for biota to survive was to retreat to refugia of more hospitable terrain, where at least some vegetation survived, and dragonflies could complete their larval life. In Europe, the Mediterranean basin was the most important of the refugia west of the Asian continent. There was one comparatively small western and one complex eastern refugium. The western refugium was composed of the Iberian Peninsula and the Maghreb, *i.e*., the Atlas region of North Africa, extending to the foot of the Alps, a filter rather than a barrier. This constrained the refugium to an area between mountains and seas – the Mediterranean and the Atlantic. The Apennine Peninsula presented a transitory position.

The eastern refugium was much wider and included the Balkans and, east of these, the complicated, continent-sized landmass of Asia. This included a mosaic of plateaus, lowlands, and mountains in areas such as Anatolia, the Black and Caspian Sea basins, the wettest parts of the Irano-Turanian region of Asia, South Siberia as far east as Mongolia, the Altai, and the north-western Tien Shan mountains. This region has, to date, been less well studied and is topographically diverse. Its margins form natural boundaries to the distribution of *Calopteryx*. It includes several rain-shadow systems resulting in a patchwork of localised arid zones or areas of higher rainfall. Some of these – *e.g.*, the Crimea, the Pontic, the Alborz, the Dzungarian mountains, the Altai – function as dragonfly refugia. In West Asia, the ice age was less influential than in Europe. Relict dragonfly populations here are numerous and have not yet all been documented. The latest cold episode peaked some 20 000 years ago in Europe and also somewhat earlier, being less severe in Irano-Turanian Asia. This allowed speciation processes to begin, but not to reach a stable endpoint. Countless novel phenotypes popped up and experienced introgression as stages came and went (Harrison & Larson 2014). Hybridization was common but so was extinction. During interglacials, populations of plants and animals expanded from territories that had previously fallen dry and saline, or turned into shallow seas – *e.g.*, the Adriatic Sea or large tracts of the North Sea. Especially when the climate was halfway through a glacial cycle, *Calopteryx* could benefit from rising temperatures, but sea water levels were still low enough for them to expand their range. Expansion rates may have reached a thousand kilometres per millennium, or more.

Material and methods

We examined specimens from collections and fresh material of described species and subspecies of *C. splendens* and allied taxa, which necessitated extensive travel and exchange (Appendix II). The fieldwork and collecting took more than half a century, from the mid-1960s to 2017. In the end, we arrived at a reasonable coverage and evaluation of the plethora of named taxa. We did not include Nearctic *Calopteryx,* except for two species that allowed us to roughly define the position of North American taxa in the phylogeny of the genus. We have not studied North American taxa at the species level but see that our molecular phylogenies single out American taxa in a clade that is of genus level. We invite others to investigate this problem.

We also used all published information that we could obtain, published papers as well as grey literature. We examined classical structural characters (*e.g.*, appendage morphology, wing shape, wing venation density, pronotum, ovipositor), reaching the same conclusion as our predecessors, viz. that they were of limited use. Usable traits were significantly restricted to wing patch size in males and the presence or absence of wing markings in females (androchromy, formerly called homochromy). Wing shape might also differ in a species-specific way, but this issue requires subtle analysis and does not lead to visually obvious results; a rigorous analysis requires time and the use of advanced mathematics (Sadeghi *et al.* 2009, 2010; Sadeghi & Dumont 2014).

One of the stumbling blocks to understanding *Calopteryx* was the recurrent occurrence of populations with similar phenotypes in widely distant locations. In our view, this reflects effects of the Pleistocene glacial/interglacial climate and the expanding and contracting of dragonfly ranges controlled by this. Finally, while past authors have studied parts of *C. splendens*' zoogeography, we discuss the topic over its full geographic range. For all practical purposes, this corresponds to the West Palaearctic (Fig. 1).

We examined and assessed museum specimens with published descriptions, and in one case examined and measured the male wing marking of a series of animals in an area extending from the south-west of France to Belgium; for details of the methods and results, see Mertens *et al.* (1992) and Dumont *et al.* (1993). The conclusions from these studies are expanded here. A wing patch index of zero means no marking at all, *e.g.*, in the taxa *C. splendens waterstoni* and *C. splendens hyalina*, while a wing patch index of 1 signifies wing fully pigmented. This latter condition is not found in *C. splendens* but occurs in some *C. virgo* males. In *C. splendens ancilla*, patch indices are between 0.7 and 0.8. We accept that the glacial cycle lasted for about 150–160 000 years and that wing patch size is a factor in considering relatedness even if not resulting in a taxon name.

Wing and geographic insights were checked against molecular data, involving fragments of DNA, one nuclear, the ITS1 and 2 of the rDNA operon, and the so-called barcoding fragment of the COI (cytochrome oxidase I) mitochondrial gene. Both have been extensively used as markers in the last two decades, and the methodology for acquiring sequences is now standard, with a high percentage of successful DNA manipulation. For a full explanation of the procedures for extraction, purification, alignment, and sequencing of the fragments of DNA, we refer to earlier papers by our team (Weekers *et al.* 2001; Dumont *et al.* 2010; Guan *et al.* 2013). Both markers were useful but not perfect. There was, for example, an inconsistency in *C. xanthostoma,* where COI has not evolved as in most species, but other characters support full species status (see below). In addition to our current genetic analysis, we included codes from GenBank (Benson *et al.* 2008).

For each specimen, a 1.0 mm section of a leg was transferred to a tube with 20 µl 0,05N NaOH and 2 µl 5 % Tween 20. This was heated for 15 minutes at 95°C and cooled on ice. 100 µl sterile water was added to the tube and mixed. 1 or 2µl of this solution was used in a PCR reaction. We amplified and sequenced the barcoding fragment of the cytochrome c oxidase subunit I (COI gene) and the entire spacer region comprising the ITS 1 intergenic spacer, the conserved 5.8S gene, and the ITS 2, using the PCR. Primers used for PCR were CO1490F (5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3') and CO2198R (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3') (Folmer *et al.* 1994) for the COI fragment. Cycle conditions were 95°C for 3 min followed by 45 cycles of 95°C for 30 sec, 48°C for 30 sec, and 72°C for 1 min. Primers used for amplifying the rDNA fragment are Vrain2F (5'-CTT TGT ACA CAC CGC CCG TCG CT-3') and 28R1 (5'-TGA TAT GCT TAA NTT CAG CGG GT-3'). Cycle conditions were 95°C for 3 min followed by 45 cycles of 95°C for 30 sec, 54°C for 30 sec, and 72°C for 1 min. PCR products were sequenced on an ABI 3130XL automatic sequencer from Applied Biosystems with the Big Dye 3.1 kit according to manufacturer instructions.

Tree building and age estimation

Alignments were made with Mafft (Katoh *et al.* 2019). The model of DNA evolution best fitting the data was determined with JModeltest version

2.1.10 (Darriba *et al.* 2012). Based on the Bayesian information criteria (BIC), the best model was chosen for inference in Mr Bayes 3.2.7a (Ronquist *et al.* 2012). The settings were: 10 million generations, a sample frequency of 1 000, and a burn-in value of 5 000 trees.

Haplotype maps use the same information as trees but are more visual. They were built in PopART with the TCS network interference method using the COI alignment (Leigh & Bryant 2015).

The genetic distance between individuals was estimated by the Kimura 2-parameter (K2-P) expressed as a percentage. A distance matrix was constructed in Mega X (Kumar *et al.* 2018). Transitions and transversions were included, uniform rates among sites and gaps were pairwise deleted. The roughly estimated time to full speciation requires a genetic distance of about 3 % or more.

Because we were interested in absolute times, crude as they may be, a divergent time estimate with error bars was obtained using Beast v. 2.7.3 following the tutorial 'Calibrated Species Trees' by H.A. Ogilvie (https://taming-the-beast.org). In the 'Taxon sets tab' the species were grouped in species groups and the *C. splendens* species group was divided in *C. splendens* and *C. xanthostoma*. In the tab 'Priors' all *Calopteryx* species were selected as monophyletic groups with an offset of 20 M years.

Results

Bayesian estimates of the phylogeny of Palaearctic *Calopteryx* species as well as related Asian genera (*Atrocalopteryx*, *Matrona*, etc.) are shown in Figures 2 and 3. A time-calibrated estimate is provided in Figure 4. A striking young age and overwhelming polytomy of the *C. splendens*-group was recovered by all methods.

The haplotype map shows a close clustering of *splendens*-like taxa, so close that in most cases even no subspecies can be upheld (Fig. 5). Save the cases that will be discussed below, all specimens, however morphologically different, must fall under the name *C. splendens splendens.*

Two species allowed us to roughly define the position of North American taxa in the phylogeny of the genus and we find that they are not *Calopteryx* but a genus in its own right.

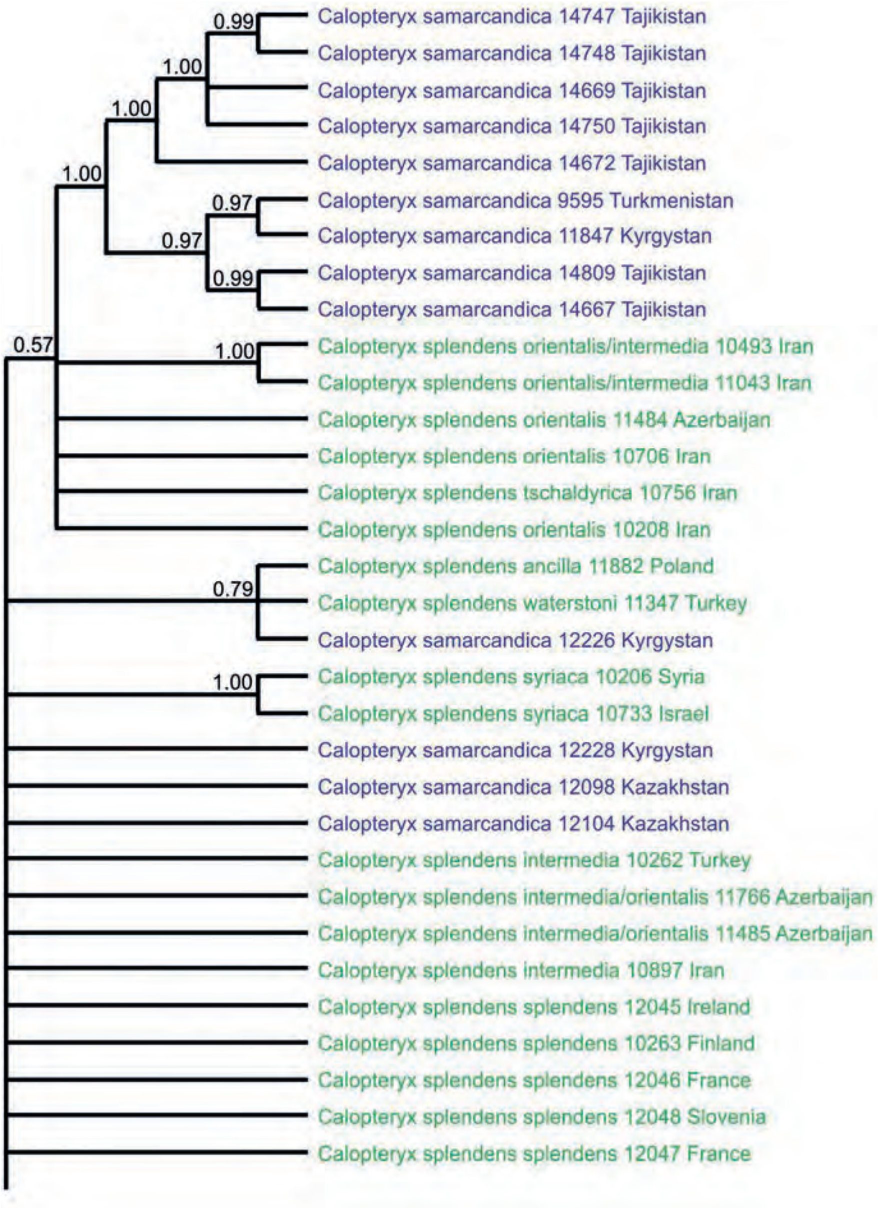

Figure 2. cont.

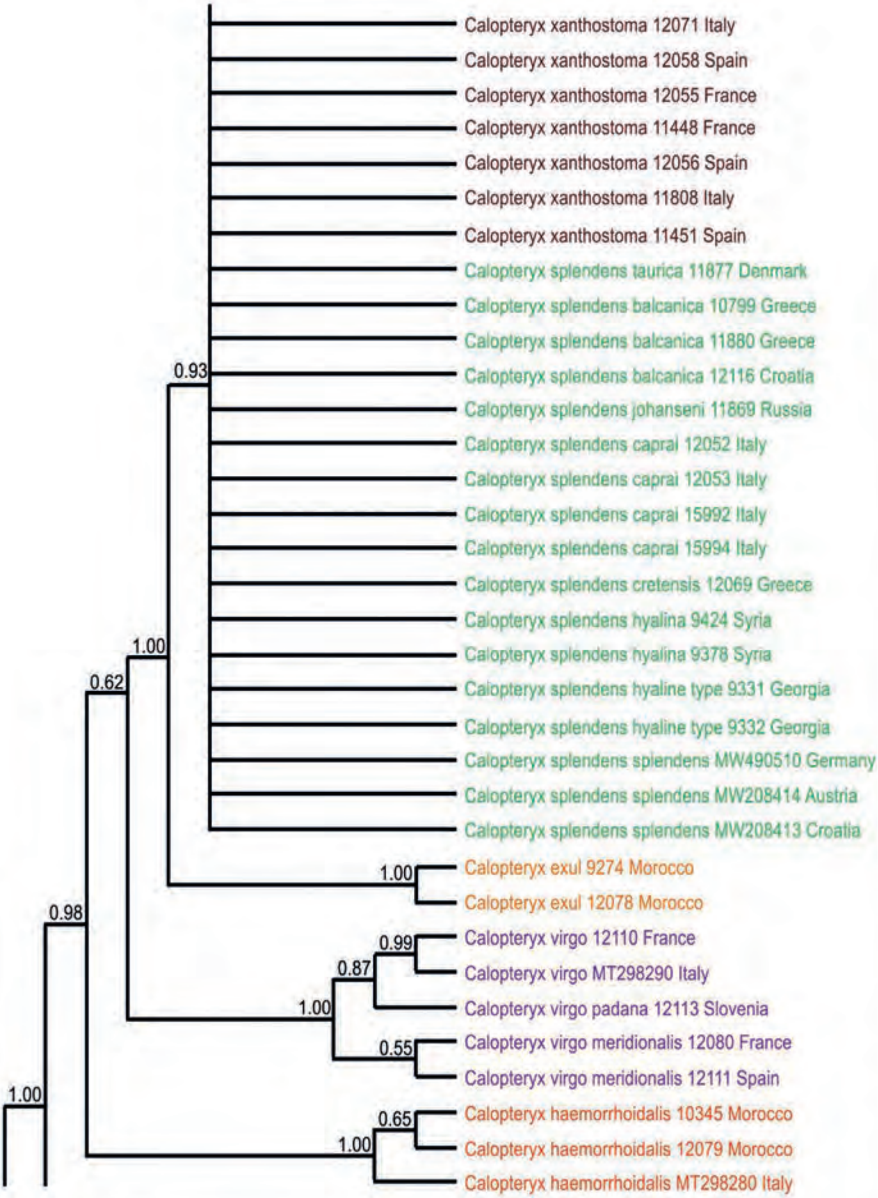

Figure 2. cont.

We use the term 'morph', a neutral designator to identify animals with large intrapopulation, often more or less geographically defined variation in male wing markings, yet no claim to taxon rank because upon contact with a subspecies or species its characters are rapidly lost through hybridization. 'Morph' allows local morphologies to be given names without taxonomic issues.

Discussion

The most widespread *Calopteryx splendens* morph, which we call morph *ancilla/intermedia*, has extensive wing marking reaching from the wing tip almost to the wing base, with androchrome females being common (Figs 6, 7). The type locality, Akbès on the Turkish-Syrian border, has been widely cited for *C. splendens intermedia* Selys, 1887, which was called a subspecies

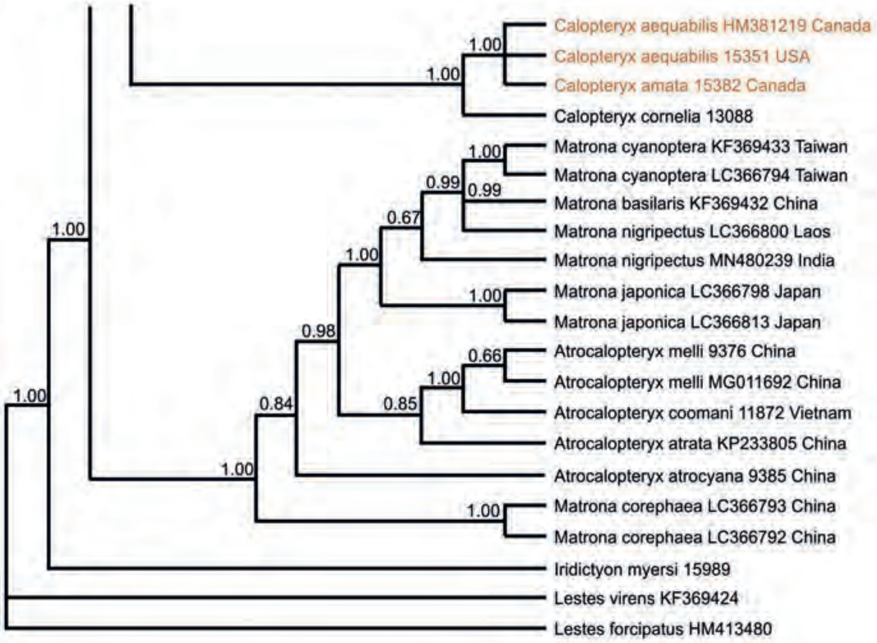

Figure 2. Bayesian phylogenetic tree of *Calopteryx splendens* and related species and genera using the so-called barcoding fragment of the Mt DNA COI-gene. The tree probably contains some unrecognized hybrids.

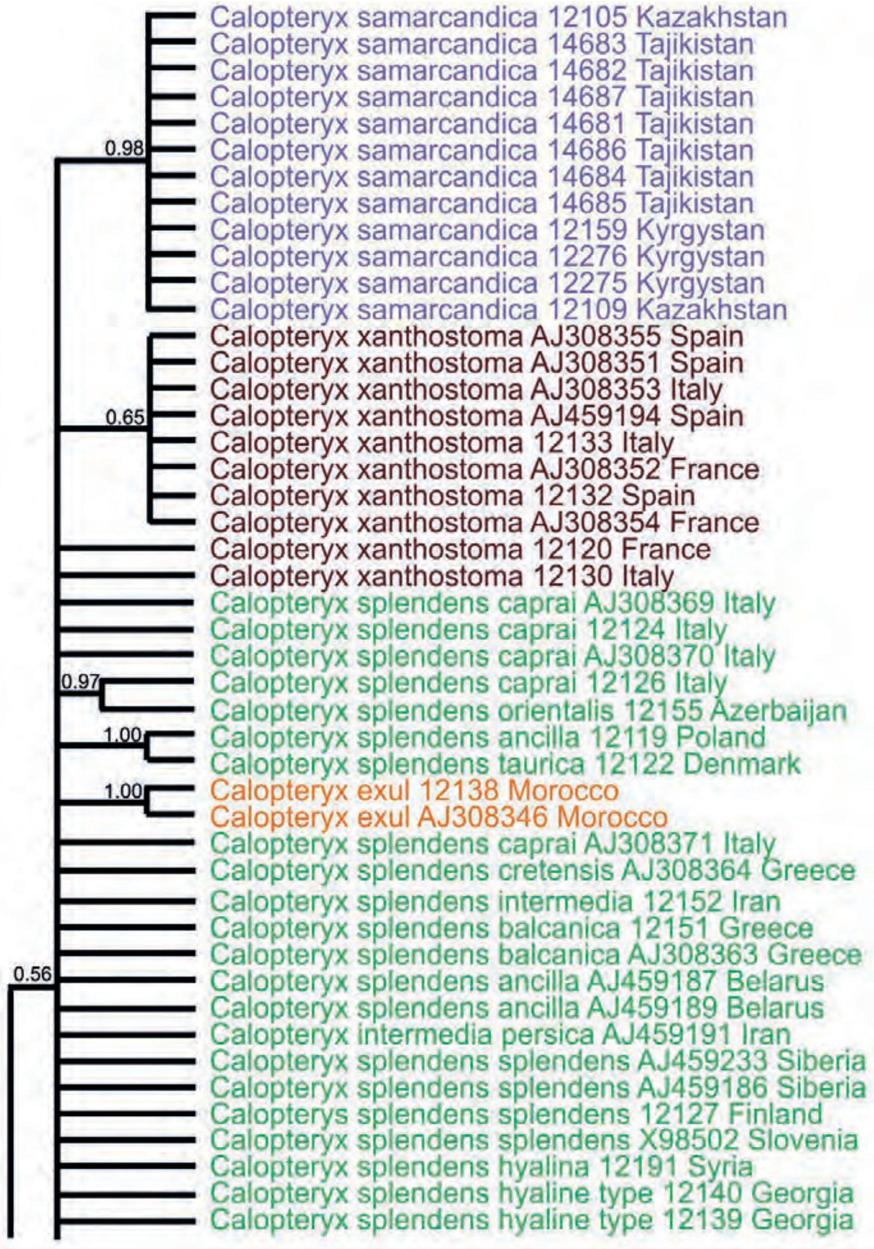

Figure 3. cont.

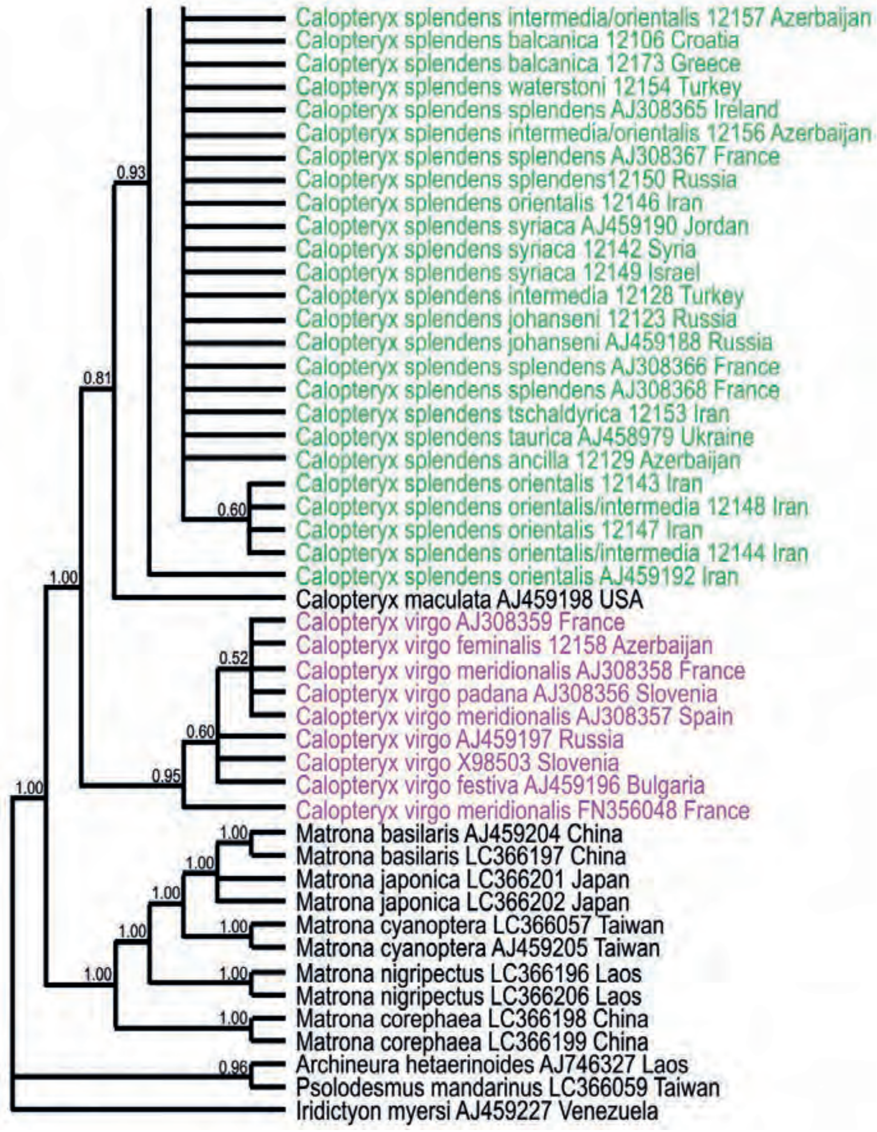

Figure 3. Bayesian phylogenetic tree of *Calopteryx splendens* and related species and genera using the nuclear rRNA operon sequences of the ITS1 and 2 (full sequence). The tree may contain unrecognized hybrids. Almost all subspecies of *C. splendens* are unsupported. *Calopteryx exul*, *C. samarcandica*, and C. *xanthosoma* are supported but not strongly, confirming their recent origin.

or even a species until molecular support was found to be absent. However, this taxon is identical with the earlier named *C. splendens ancilla* Hagen in Selys, 1853, from Königsberg (Kaliningrad) in the Baltics. Figure 8 shows an a*ncilla* male in the type series of the Selys collection at Brussels. However, other specimens in this series have hyaline fringes at the wing tip and are intermediate, thus hybrids with typical *splendens*.

Calopteryx splendens was described – as *Libellula splendens* – by Moses Harris from southern England in a bilingual English-French paper (Harris 1776–[1780]). The date of publication was long given as 1782, until Hämäläinen (2008) showed that this was two years out and *Calopteryx splendens* (Harris, 1780) was the correct date. The description is short, but the figures include a male with hyaline wing tip and a wing band extending basad of the nodus (Fig. 9).

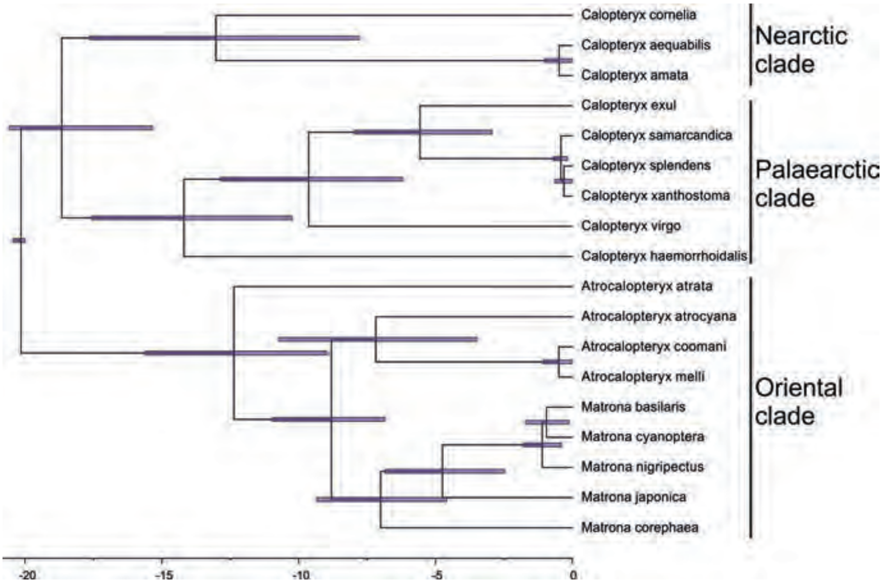

Figure 4. Time-calibrated map with a time offset of 20 million years, for several calopterygine genera. The Nearctic clade might be accorded separate genus status. *Calopteryx* is recovered as monophyletic. The East and Southeast Asian genera *Atrocalopteryx* and *Matrona* seem paraphyletic and need further study.

We now know that in the British Isles populations the wing bands exhibit a size gradient from south to north, becoming larger in the north, consistent with Bergmann's rule (Upton *et al.* 2016). Wing markings reduced to a discrete ovate patch are not rare. Further in this article, we will explore the meaning of wing spot size and form. Other, more extreme phenotypes exist, which will be discussed below.

Additionally, the trees reveal the following: three members of the *C. splendens*-group (*exul, xanthostoma,* and *samarcandica*) have escaped from the

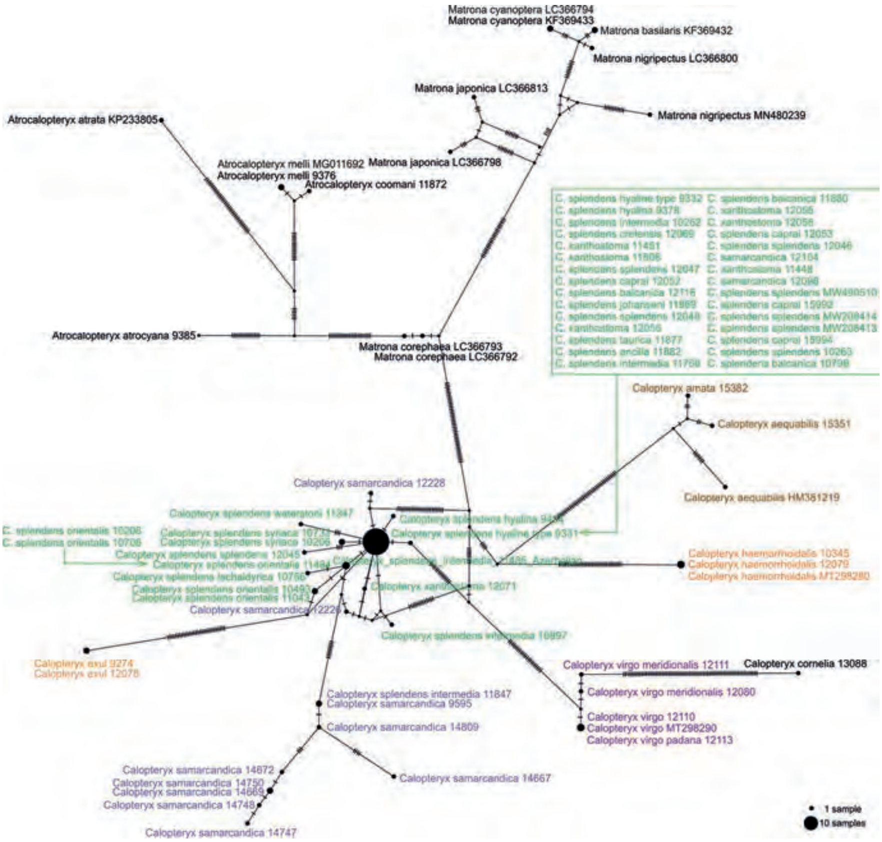

Figure 5. Haplotype map for COI of *Calopteryx* and related genera. The *C. splendens*-group shows little evolution and seems to include *C. xanthostoma*. Only *C. exul* and *C. samarcandica* stand out and are supported as distinct species.

polytomy of the COI and/or ITS trees and have an age of three or four glacial cycles, or more in the case of *C. exul* (Fig. 10). These three are considered to have evolved beyond the speciation threshold. The taxa *orientalis* and *syriaca* (Fig. 11) are borderline cases, *en route* to speciation, but best

Figure 6. Above – male, below – female of *Calopteryx splendens* morph *intermedia* from southern Turkey, Muratbeyli Amik Golu, May 1988. Coll. HJD, Ghent

considered subspecies of *splendens*, with moderate molecular support. Almost all other names in the tree turn out to be of late Würm glacial age, generally too short for even subspecies to evolve. They could therefore be as young as 20 000 years or less, and they all fall in the synonymy of *C. s. splendens*. The couple *orientalis* and *syriaca* (Fig. 11) is morphologically similar but is broadly separated by the Anatolian land mass and well isolated in the Caspian depression and African Rift, respectively. Their common ancestor must have occupied the central Anatolian plateau during a not too recent interglacial. We estimate it at some 200 000 years BP. One difference between them is that androchrome females are common in *orientalis* but rare in *syriaca*, and if present, reduced to a faint brownish mark at the apex of the hind wing (Dumont 1991: fig. 30). According to observations by T. Schneider, this mark is an effect of ageing. We consider this sufficient support to classify both as distinct subspecies.

Figure 7. A series of the *Calopteryx splendens* morph *ancilla*/*intermedia* (below, left – two males, right – four females) compared to syntopic *C. splendens splendens* (above – three males). Topar River, Bola-Topar, Balkhash Region, Kazakhstan, 354 m, leg. SNB, 15.–16.vi.2005. Coll. SNB, Novosibirsk

Calopteryx exul is endemic to the Maghreb and the only distinct breakaway species older than a million years. With genetic distances of 7.10–8.19 %, a Pliocene origin is accepted (Appendix I). It has narrow hyaline wings, a straight and more rapid flight than *C. splendens*, and tends to live a solitary life, although rarely aggregations can be seen, as in most *C. splendens* forms. Two other forms without wing markings are the morphs *waterstoni* and

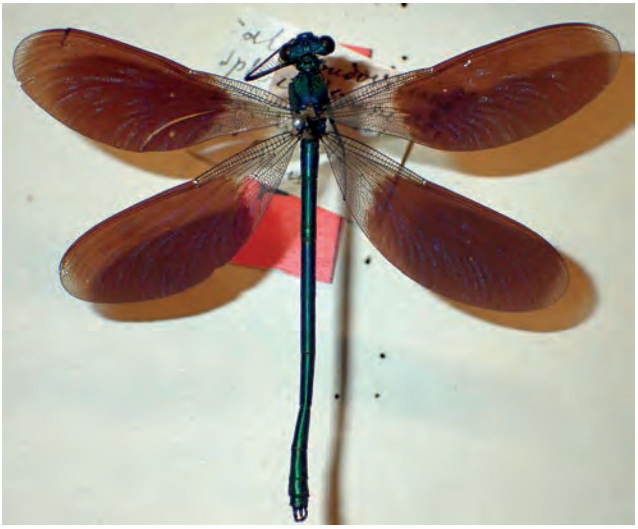

Figure 8. Male syntype of *Calopteryx splendens ancilla* from Königsberg (Kaliningrad), Baltics; there are about ten specimens in the Selys collection at IRSN Brussels. Photo: HJD

Figure 9. Picture detail in the original description of *Calopteryx splendens* (Harris 1780, 1st ed.: Plate XXX) depicting the male type specimen. Photo: MH

Figure 10. A rare occasion: a social gathering of the chiefly solitary species, *Calopteryx exul*. Bizerte, Tunisia. Photo (v.2009): TS

Figure 11. Above – two males of *Calopteryx splendens orientalis* from the border zone Iran-Azerbaijan; below – *C. splendens syriaca* male from river Jordan. Coll. HJD, Ghent

Figure 12. Left – *Calopteryx exul*, male from Tunisia; centre (female) and right (male) – *C. splendens* morph *hyalina* from northern Syria. Coll. HJD, Ghent

Figure 13. Left – *Calopteryx splendens* morph *hyalina*, Munjez, northern Lebanon; right – *C. splendens* morph *waterstoni*, Sürmene, Trabzon Province, northern Turkey. Coll. TS, Berlin

hyalina (Figs 12, 13). They cannot be distinguished by skeletal morphology from other members of the *C. splendens*-group and promptly hybridize with them should they encounter each other (Fig. 14). *Calopteryx splendens* does not co-occur with *C. exul*, and thus no hybrids are known.

Calopteryx samarcandica adults have a diffuse deep brown wing with iridescent blue veins (Fig. 15). The wing colour becomes darker with age and may form two distinct patches. At emergence, and during the first week of adulthood, the wings are hyaline. This prompted the description of *Calo-*

Figure 14. Three specimens from the hybrid population of *Calopteryx splendens* morph *waterstoni* at Gorele, Black Sea coast, Turkey. Above – true morph *waterstoni*, centre and below – hybrids with other members of the *C. splendens*-group. Coll. HJD, Ghent

pteryx unicolor by Bartenev (1912), clearly a synonym of *samarcandica.* Its range is limited but well defined (Fig. 16). It takes a bite out of the vast territory of *C. s. splendens* (Fig. 1) and fans out with some hybridization with *splendens* on the north flank of the foothills of Kazakhstan, Tajikistan, and Uzbekistan. That contact may or may not be an artefact of 20th century irrigation canal construction in Central Asia (Góral *et al.* 2023). In our team, S. Borisov surveyed the region, where *splendens* and *samarcandica* co-exist for many years and found that the two rarely co-occur: mixed populations exhibit rather strict segregation, apparently maintained by different habitat choices. *Calopteryx splendens* morph *ancilla/intermedia* is found in turbid irrigation canals but not on spring rivers. On the other hand, *C. samarcandic*a is found on short rivers that spring from lakes or springs in the mountains and have clear and pure water. It is quite possible that *C. splendens* is an invasive species in Central Asia that was introduced not more than a century ago due to irrigation.

Figure 15. Males and females of *Calopteryx samarcandica*. Kodara village, southwestern Tajikistan, leg. SNB, 29.iv.2019. Above – nine males; below – six females. Coll. SNB, Novosibirsk

The genetic distance between the two competing species is around 3 %, at the lower end of the accepted species level (Appendix I). At such values, hybridization should still be relatively easy, and indeed, in series of animals caught in marginal biotopes, specimens show intermediate characters with blue rather than golden body sheens and wing marking shaped as in morph *ancilla*/*intermedia*. However, the genetic distance seems to be sufficient to limit introgression in *C. samarcandica*.

What about *C. splendens splendens*? In Figure 17 we selected a few examples to illustrate the extent of variation in wing marking, which shows strong hybridization of animals from the eastern part of the range.

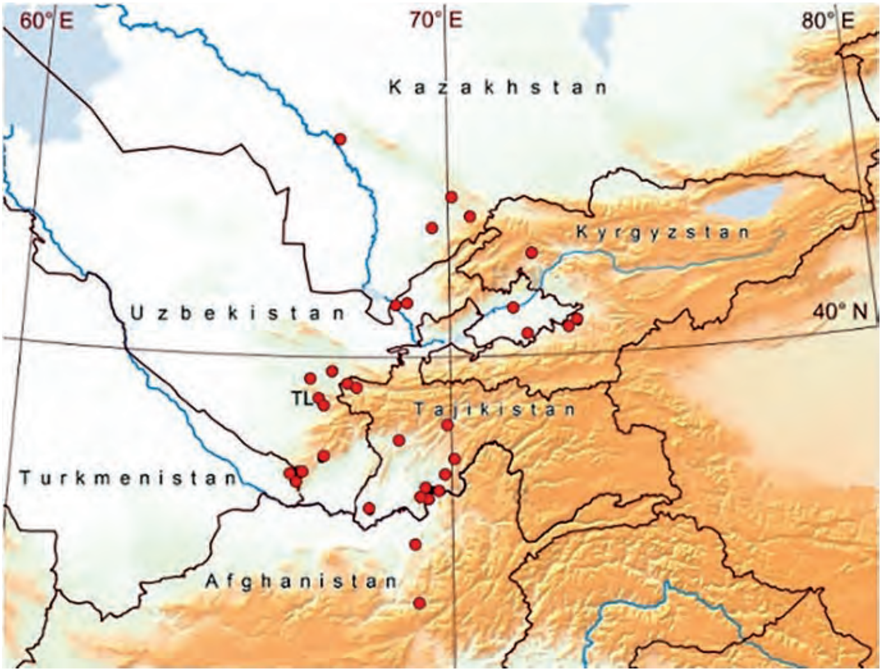

Figure 16. Map showing localities of *Calopteryx samarcandica* in Kazakhstan, Uzbekistan, Turkmenistan, Kyrgyzstan, Tajikistan, and Afghanistan. The range covers the mountains and foothills of the western Tien Shan, the western part of the Pamir-Alai and the northern macroslope of the Hindu Kush; TL – type locality. Hybridization with *C. splendens* is supposed to be limited to the northernmost populations. The localities are given according to the data of Boudot *et al*. (2021).

A last member of the *splendens* group with known but limited hybridization is *C. xanthostoma*, an endemic of the Iberian Peninsula and the Maghreb. It still co-occurs with its distant relatives *C. virgo* and *C. haemorrhoidalis,* but hybrids are rare. *Calopteryx xanthostoma* was recovered as distinct by its ITS but not by COI, a case of disagreement between DNA fragments. We accept its status as distinct species because it rarely hybridizes, has no androchrome females, and its wing marking reaches the tip of the wing (Fig. 18). Also, its wing marking only appears several days after emergence. Its range, the Iberian Peninsula – Maghrebi populations went extinct in the 19th century – is also interesting.

Figure 17. Four specimens of *Calopteryx splendens splendens* from the eastern part of the range, with phenotypes that are ‘typical’ *splendens*. Above – Semikorakovsk, river Don, Rostov region, Russia; central two specimens – Kyzyl-Orda, lower reaches of river Syr Darya, southern Kazakhstan; below – Orenburg, river Ural, Russia. Coll. HJD, Ghent

All calopterygid taxa with greater genetic distance to *C. splendens* than 3 % are regarded here as belonging to separate genera. Examples in Asia are *Atrocalopteryx* and *Matrona*. As the trees show, some extra work will be required to deal with a possible paraphyletic status of these genera. North American species are clearly not *Calopteryx* either, at least in the sense of the present paper. They require a formal generic definition. Finally, the Japanese *Calopteryx cornelia* is not a *Calopteryx*. As can be seen in Figure 2, *C. cornelia* is in the same clade as the Nearctic *Calopteryx* species. Two genus-group names are available for the members of this clade: *Sylphis* Hagen in Selys, 1853, with *Calopteryx angustipennis* (Selys, 1853) as its type species, and *Anaciagrion* Kennedy, 1920, with *Calopteryx cornelia* Selys, 1853, as its type species.

Figure 18. *Calopteryx xanthostoma*, La Roque-sur-Cèze, Gard, France. Left – young male; right – middle-aged male; below – fully adult and coloured male. Coll. HJD, Ghent

More on hybridization and speciation

Interspecies hybrids in Odonata have long been believed to be rare. In contrast, we now find them virtually everywhere. But the existence of hybrids is not sufficient to invalidate the recognition of a species. We are sympathetic to the concept of Dubois (1988) that not the species, but the genus is the fundamental unit of taxonomy, at least in some cases. This applies when the genus is a collection of all species that can be hybridized, by whatever means. Hybridization is a natural process, co-occurring with another natural process – speciation. In recent decades hybrids have been discovered in almost all animal groups. In dragonflies, they were considered exceptional until the late 20th century (Bick & Bick 1981). Asahina (1974) first offered evidence of hybrids with morphology intermediate between parents. Such hybrids are easy to find, but hybrids with parental phenotype remained hidden until DNA-based techniques permitted a direct look at individual genomes.

Speciation is a dynamic process. The more it advances, the greater the genetic gap with its relatives and the more difficult hybridization becomes, as stated earlier. But the road, *i.e.*, the number of mutations required, is long and on the way many things can go wrong. Ongoing speciation may be aborted or at least interrupted if the environment changes, as when climate change occurs. It is believed that one full glacial cycle takes around 160 000 years, so up to three or four of them are required for a prospective taxon to reach a speciation point of no return (a minimum genetic distance of *ca* 3 %). This may or may not mean full speciation but should be sufficient to resist introgression and allow a population to reach full species status eventually. Surely there are numerous false starts that end in introgression or extinction. Further, we know most about the most recent glaciation, the Würm (also known as Weichsel or Wisconsin), and considerably less about older glacial cycles.

As we have seen, speciation's best chances of success are in favourable environments provided by refugia. For Calopterygidae these include the leeward side of mountain chains, oriented such that water draining from them puts dragonfly populations in local zones of precipitation, causing prolonged isolation. In such environments, populations may have the best chance of approaching the million years of age in isolation suggested as necessary by Uyeda *et al.* (2011).

Decisive features that make refugia favourable to *Calopteryx* are temperature and humidity. Atmospheric temperatures just south of the glaciers were up to 4–6°C colder during a pleniglacial than during an interglacial. An enormous amount of water was thereby sequestered in glaciers, and the level of the Atlantic and Mediterranean fell by up to 130 m. Even in parts of Europe not under ice, life was severely constrained and retreated to the shores of the Mediterranean Sea. During interglacials, as temperatures rose, *Calopteryx* morphs began dispersing from their sanctuaries, one after the other. The most cold-adapted form came first and reached farthest north. This, in our opinion, is the key to understanding the current distribution patterns. The available evidence supports the idea that there were at least two ‘streams’ of dispersing animals. A cold-adapted strain was composed of animals without any wing marking, thus the phenotype *hyalina/waterstoni.* They travelled from a refugium, perhaps near the margin of the Black Sea, which they still partly occupy. A warm-adapted wave came later and probably looked like morph *ancilla/intermedia.* Warm- and cold-adapted are relative terms: The minimum temperature tolerance of both waves may not have differed by more than a couple of degrees Celsius.

The age of the waves is hard to give exactly, but the lack of DNA evolution (none or a maximum of two base-pairs difference between any two DNA molecules) argues for the late Würm, thus the first wave started moving during early deglaciation, perhaps 20 000–18 000 YBP. The second wave, more warm-adapted, seems to have moved relatively late, not more than 10 000–15 000 YBP. It also seems to have been competitively dominant and erased much of the cold wave. Our molecular markers are not well suited for picking up such early phases of evolution, but these had a visual aspect: the two extreme morphs *ancilla/intermedia* (with large male wing patch) and *hyalina/waterstoni* (no wing marking at all). On meeting, these two waves freely hybridized, producing today’s rich panoply of phenotypes. Hybrids closer to *waterstoni* have wing markings not touching the wing margin. They are classified here with group *waterstoni.* Specimens with dark patches touching the wing rims and larger in size are placed with *ancilla/intermedia.* Taxonomic logic dictates that both be called *Calopteryx splendens splendens.*

Origins and postglacial expansion

Postglacial expansion must have had a point of origin but pinpointing it is speculative. Where the occupation of new territories began and why some populations expanded and others, like *orientalis*, limited to the southern fringe of the Caspian Sea and the Kopet Dag mountain range, did not, is not known. Fact is, however, that *C. s. orientalis* is firmly established in its range. Working with *Calopteryx splendens s.l.* we finally arrived at a model with two main players, morphologically distinct but genetically similar, biogeographically partly overlapping and competing with each other. We also have little idea of previous, pre-Würmian evolution of the two basal forms of the *C. splendens*-group. Thus, we place the origin of cold-adapted morph *waterstoni* at the southern rim of the Black Sea, the only zone where it is still locally common. It certainly was more widespread in the past, as supported by the presence of hybrid populations of morph *taurica* in the Crimea mountains and around the headwaters of the Danube. This pattern, morph *waterstoni* and close hybrids surviving at the boundaries of morph *ancilla/intermedia* and at higher elevations, recurs as far as Afghanistan: Schmidt (1961) mentions four specimens, correctly identified as *C. splendens splendens*, collected at »Bareki« near Urozgan, at 2 500 m a.s.l.

The origin of the warm-adapted morph *ancilla/intermedia* was further east, an area from the Zagros Mountains in Iran to the north-west as far as Hatay province, Turkey. From there, rich populations of robust *ancilla/intermedia* occupy a wide region, with complex high mountain barriers as far as the Himalayas preventing further eastward expansion. The warm-adapted wave split into branches passing north and south of the Lut-Kevir deserts of Iran. These branches approached each other again at their western end and may have made secondary contact in the Baltics. Morph *ancilla/intermedia* may have displaced *waterstoni* up to some unspecified elevation – around 2 500 m a.s.l. as claimed by Schmidt (1961) for *C. s. splendens* in Afghanistan.

Forms with reduced or no wing markings have been described under 'local' names (*taurica, tschaldirica, erevanense, njuja*), the latter from the middle Lena region in Siberia (Fig. 19). It is unclear where the borders of its range are. All these names apply to postglacial populations, too young to have taxonomic meaning beyond the fact that they belong to the *C. s.*

splendens-complex. Authors may feel free to use such names in a geographic, population dynamics, or biogeographic sense, as done in this paper. They do not denote formal species-group taxa and are here used as an *aide memoire*.

Novel hybrid phenotypes and the long cline from Zagros to the west

At Reyhanli in Hatay province, Turkey, near the tip of the African Rift valley, a hybrid population similar to the one at Gorele, the place on the Black Sea coast where *waterstoni* meets *splendens,* is found. The difference is that the mix here is one between morph*s ancilla*/*intermedia* and *hyalina*, which occurs in typical *waterstoni* form on small rivers draining the Nur hills to the Mediterranean. Many have intermediate or paternal-sized wing patches,

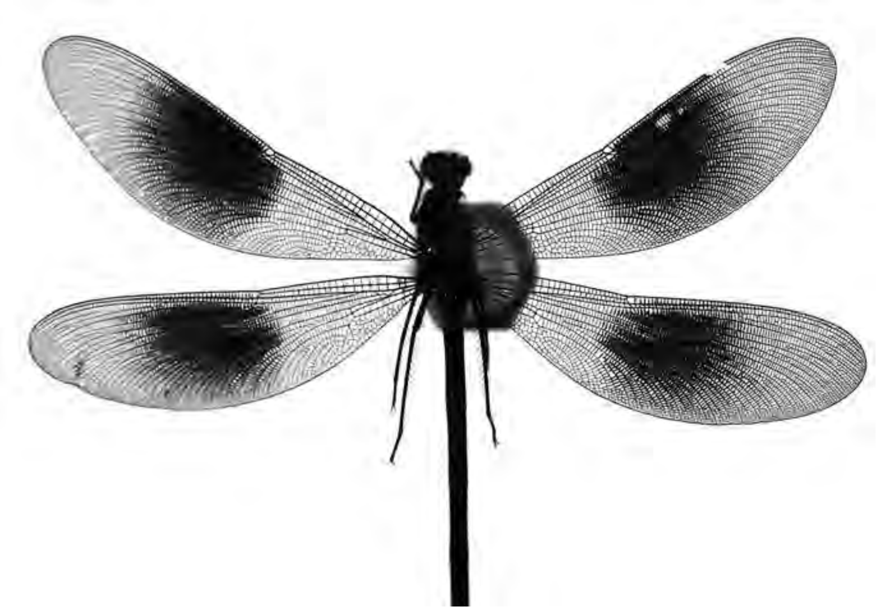

Figure 19. A male of *Calopteryx splendens* morph *taurica*, with reduced wing markings, especially in the hind wings, from Simferopol, Crimea. Similar populations, not physically in touch with each other, occur in central England, Ireland, Denmark, south-eastern Anatolia (Kars-Cildir lake area; morph *tschaldirica*), Armenia (morph *erevanense*), and in the south-west of Yakutia, Russia (morph *njuja*). Coll. HJD, Ghent

but some had apical colour smears seen nowhere else, and both Schmidt (1954) and Buchholtz (1955) discovered and named them ssp. *pseudosyriaca*. However, this name is considered not available as it was designated as a synonym in the same paper where it was introduced (Hämäläinen & Fliedner 2022: 127–128). We resampled the area twice (HJD 1977, 1988) and in the field there was no evidence of preferential mating with respect to wing patch shape or size. Morph *pseudosyriaca* was rare, but the full range of intermediate morphologies was present. Unfortunately, no further research on this or adjacent populations has been carried out.

Clines in wing patch area are continuous hybrid zones, gradually changing over hundreds of kilometres, and the 'steep' Gorele and Reyhanli clines represent the tip of such mega-clinal structures that may date back to the early Holocene. There probably exist more of them, still undiscovered. The longest known cline extends from the Zagros Mountains in Iran, via east Iraq and Mediterranean Turkey, the *terra typica* of the morph *intermedia.*

In Hatay province in southern Turkey lives pure morph *intermedia* – which has a phenotype with little variability. The wing patch begins basally at about the midpoint between the wing base and nodus and reaches the wing tips apically; sometimes all females are androchrome. As soon as the Holocene climate permitted, this cline began to expand. It dispersed in westerly direction along the coast of Anatolia, crossing the then narrow Aegean Sea, and reached Greece and the Balkans. The phenotypes now became variable in Croatia. Male wing tips often are hyaline. The females are a mix of androchromes and hyalines. In the east, and on the high plateau of eastern Anatolia, there is a continental climate with severe cold at the cities of Kars and Agri, and lake Cildir, where winter is the longest season of the year.

Here, the wing patch size tends to remain small to absent but is likely composed of several different genetic inputs. Large patches pop up again in the low-elevation Iranian Azerbaijan province, where morph *intermedia* appears again. In between, a myriad of intermediate forms is found. Unravelling the genetic entanglements here is hoped to take place in the near future. The phylogenetic trees, however, suggest once more that all relationships are very close.

On the Mediterranean side of Anatolia, at the foot of the Taurus mountains, *C. splendens* crossed the Aegean Sea towards Crete and settled on several reachable islands. A poorly defined subspecies, *cretensis*, was described from Crete (Pongrácz 1911). We synonymize it with *C. s. splendens,* along with the morph *tschaldirica*, originally from the north-eastern Anatolian plateau. At the time most of the Adriatic Sea was dry and presented no obstacle for *Calopteryx* and other dragonflies. In south-central Italy, populations of the phenotype *ancilla/intermedia* with androchromy were described by C. Conci as ssp. *caprai* (Conci & Nielsen 1956) and synonymised later with *ancilla* (Lohmann 1992). They are best considered a morph; populations even further north, described as »var. *faivrei*« (an infrasubspecific name for the androchrome female form) with variable hyaline wing tips, inhabit south-western France (Lacroix 1915). A rare androchrome female of this morph from Saint-Jean d'Angély is shown in Figure 20. A general observation is that as the wing patch contracts, androchrome females become rarer or disappear entirely. As far as we know, a single specimen has been captured near Marseille in recent times (Papazian 1995). The northernmost androchromes known occur are on the rivers Boutonne and Charente. These animals are considered to be *C. s. splendens.*

According to Lieftinck (1925), populations in the north of the Netherlands include individuals with the hind wing patch beginning to detach from the wing margin, such that it is not a band but an almost circular patch, without any contact with the wing margin. This is the point where we classify populations as part of the *hyalina/waterstoni* phenogroup, the cold-adapted group. The British Isles are of interest since they are the *terra typica* of *C. splendens,* common in southern England but rare in the midlands and the north. Numerous photographs of males from the British Isles on the Internet confirm the rather common presence of such 'circular' wing patches in England, beside individuals with 'classical' wings confirming the genetic mix of the terra typical population. The same situation occurs in Ireland. All this suggests the type population of *splendens* is hybrid and finds a logical place in the long row of phenotypes between morph *hyalina/waterstoni* and morph *ancilla/intermedia.*

Moving even further north, in Jutland as well as in southern Germany near the headwaters of the river Danube near Donaueschingen, the upper Danube, along the river Vils in Bavaria, and North Tyrol in Austria, Gorele-like populations occur, lacking a normal wing patch and thus identical to morph *hyalina/waterstoni*. The first mention of such animals was by Ris (1918), but full variation was photographed by Seidenbusch (1994, 1997). It is possible that this phenogroup is confined to the headwaters of the Danube and brings a natural end to a long cline. Nevertheless, mapping is required to sort out the details of the situation because in this area of East Germany, Poland, and Baltic states, the situation again reverses: Forms with a broad wing patch and androchrome females re-appear (Fig. 21). These merge with animals from Russia and extend into Siberia where they assume the wide-ranging *johanseni* phenotype. There may be a gap without any *Calopteryx* across Russia, but this is highly unlikely.

Figure 20. A rare androchrome female of *C. splendens* morph *faivrei,* from the river Boutonne at St-Jean-D'Angély, south-western France. Photo (viii.2012): Ch. Brochard

Androchrome females are not rare in Belarus (*e.g.*, around Pinsk; in coll. S. Gorb), as usual accompanied by males with broad wing bands, often reaching the wing tips. The Baltic States, Ukraine, and Russia, have many populations that await study. In Hagen's type series of *ancilla* (Selys 1853: 13), males have large wing patches together with narrow or no hyaline fringes (Fig. 8). How could this situation arise? The available data do not permit much factual discussion. But what we can say is that with the more humid

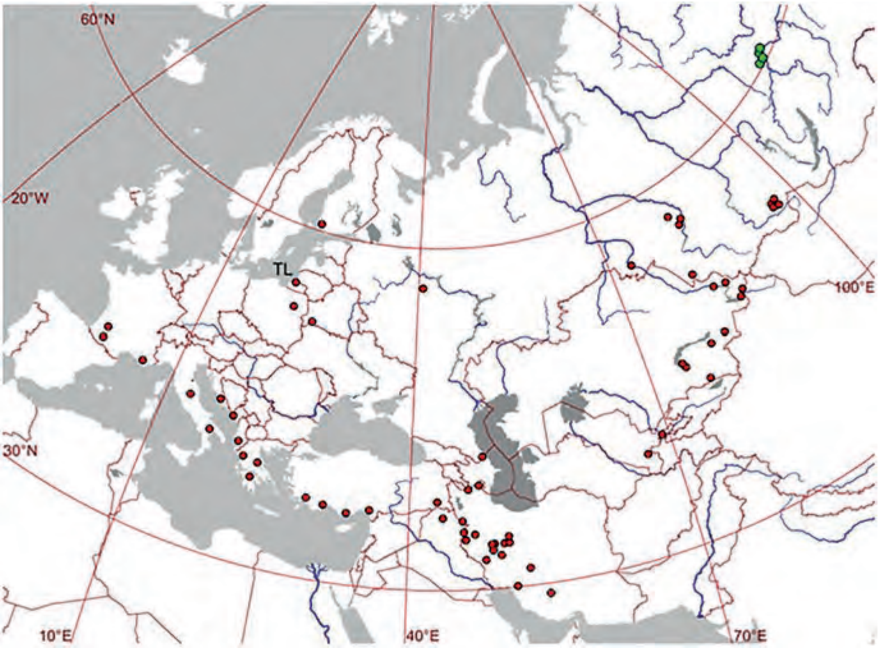

Figure 21. Records of all *Calopteryx splendens* morphs with large wing patches and androchrome females (morph *ancilla*/*intermedia*), which we assume to form a single genetic group. The U-shaped map suggests a single origin in the Zagros area in Iran, but there are objections. For example, the western and eastern tips of the U are thousands of kilometres apart in east-west direction, causing a very different climate. The rugged landscape also contributes to very different climates; finally, the time elapsed since the last glacial may be sufficient for some neo-mutations to have happened and change the thermal behavior of local *Calopteryx*. Also shown in the north-east are the records of *C. s. njuja* (● – Kosterin & Sivtseva 2009), thousands of kilometres from the nearest *waterstoni*-like population. TL – Königsberg (Kaliningrad), the type locality of *C. s. ancilla*.

mid-Holocene conditions on the plateaus of Kazakhstan, Uzbekistan, and Turkmenistan, and a Caspian Sea up to three times its present surface area, Central Asia and adjacent Siberia would have been far more attractive for dragonflies and could have been permanently inhabited. Still today, populations with androchrome females occur near Samarkand, and on the west coast of the Caucasus in Azerbaijan, north of Baku. Possibly the Baltic area is where the western cline finally reunites with the eastern cline that separated from it in Holocene times in the Lut-Kavir desert.

Calopteryx splendens and *C. xanthostoma*: a slow and a rapid cline mixing at the foot of the Alps

Mapping, preferably at 10 km intervals, is required to resolve the puzzle of the s*plendens* forms of Eurasia. Fine-grained mapping was recently carried out in Iran (Schneider & Ikemeyer 2019) and the south-east of France (Papazian *et al.* 2017). The work of Papazian *et al.* (2017) confirms *xanthostoma* and *splendens* to co-occur in Liguria (Italy) and Provence (France). During the pleniglacial, they must have survived in the same general area. Todays' situation in France has been studied in detail by M. Papazian and co-workers and is showing interspecific conflict between *xanthostoma* and *splendens* competing for each other's territory (Fig. 22). Shared occupancy of some squares still occurs but exclusion is more common and *xanthostoma* has been eliminated from a fair number of squares. Thus, *xanthostoma* is stationary or shrinking, while *splendens* continues expanding north. Indeed, *C. xanthostoma* may ultimately be eliminated completely. It used to occur in the Maghreb (Selys 1890) but has disappeared, for no obvious reason, probably as late as at the end of the 19th century. The study by Papazian *et al.* (2017) made clear that *splendens*, and *xanthostoma*, intruded into each other's habitat, and thereby may lose their identity.

A modicum of orthodox taxonomy

What names are taxonomically valid in the *C. splendens* group? The first species-group name was *C. splendens* (Harris, 1780), followed by *C. xanthostoma* (Charpentier, 1825). The description of a third taxon from Prussia, without name in the *Revue des Odonates ou libellules d'Europe* (Selys & Hagen 1850), marked the beginning of a series of scientific collaborations between Edmond de Selys Longchamps and Hermann August Hagen. Selys kept a diary that was posthumously published by Caulier-Mathy & Haesenne-Peremans (2008). It detailed his collaboration with Hagen, who had found in Prussia some androchrome females ('females with a male-like wing spot'), cited in the *Revue des Odonates* as »*variété femelle accidentelle*« (Selys & Hagen 1850: 141). Soon after this publication, he also found the male. In our copy of the diary, there is a handwritten note by Selys confirming that, from the beginning, they associated the sexes correctly. Males correspond to *ancilla*, *i.e.*, robust animals with broad wing patches, reaching the wing tip in the male, with androchrome females. The name was first introduced as »*Race prussienne* (*C. ancilla*, Hagen)« in the *Synopsis des Calo-*

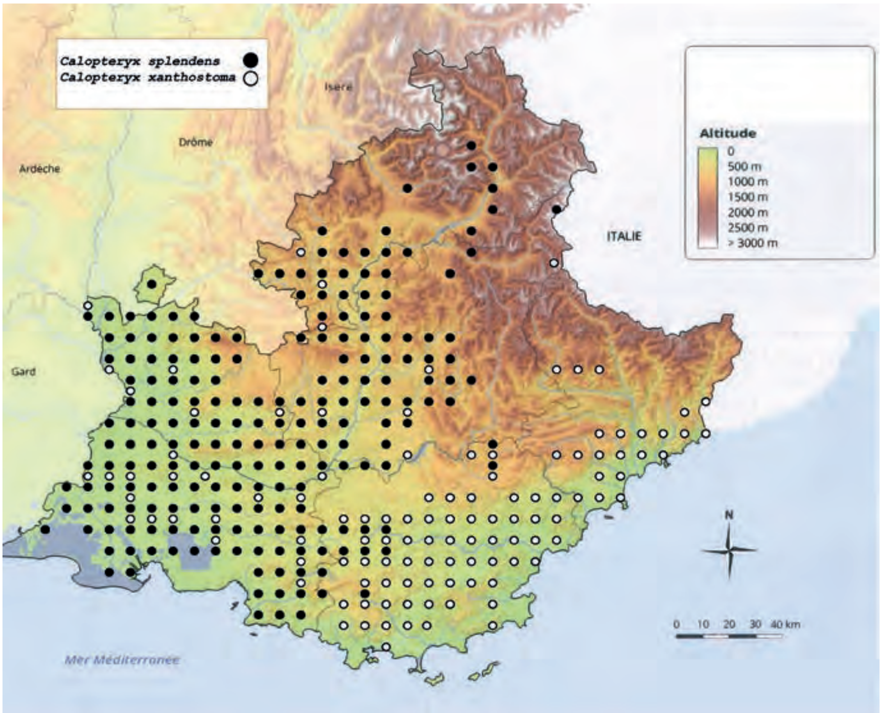

Figure 22. The meeting of the ranges of *Calopteryx splendens* (morphs s*plendens*/*intermedia*/*faivrei*) and *C. xanthostoma* at the foot of the Alps in France in the region Provence-Alpes-Côte d'Azur. Data according to Papazian *et al.* (2017).

ptérygines (Selys 1853: 13), a paper authored by Selys alone but with credit for the new taxon given to Hagen. The preceding pages have shown that *ancilla* is the most widespread phenotype of *splendens,* and mixes with a variety of other forms.

A second mention occurred in the *Monographie des Calopterygines*, another co-authored work by Selys & Hagen (1854: 39). In the text only the terms »*Race de Prusse*« and »*race prussienne*« are used, without mention of *ancilla*. The name *ancilla* then reappears in the *Odonates de l'Asie mineure* (Selys 1887: 41) as »*Race?* ANCILLA Hagen *in litt.*«, with the remark that this name had been earlier designated by Hagen in a letter for individuals from Prussia. However, similar phenotypes had by now been recorded from places such as Corfu, Dalmatia, and the Charente in France. The Balkan form would later be named *C. balcanica* Fudakowski, 1930, and the one from France *C. splendens* var. *faivrei* Lacroix, 1915. However, as *faivrei* is an infrasubspecific name for a female form, it is not a valid subspecies name.

The *Odonates de l'Asie mineure* (Selys 1887) was Selys' last major paper on *Calopteryx*, yet it escaped his notice that variation in wing patch size within a taxon could be important. He also lacked the concept of hybridization, as did many at the time. Although the 19th century was drawing to a close, the publication of Charles Darwin's seminal book "On the origin of species" (Darwin 1859) and its significance to dragonflies, as to all other animal groups, eluded Selys. Evolution was only mentioned in his diary in 1870, the year of Darwin's election as a foreign member to the Belgian academy of sciences (Caulier-Mathy & Haesenne-Peremans 2008: 825).

In the 1887 paper, Selys' final attempt to determine the differences between the taxa *xanthostoma, ancilla*, and the newcomer *intermedia*, was not successful. Illustrating Selys' confusion, almost all subspecific categories in the paper are question-marked. The taxon 'race *intermedia*' was named and briefly described from Akbès, northern Syria, within the account of »Race *xanthostoma* Charp. (*méridionale*)« (Selys 1887: 39). A photograph of the male type was provided by Schmidt (1961) and Dumont (1991). However, they suggested *intermedia* was the same as *xanthostoma*, while *ancilla* and *intermedia* are said to be easily separated from it, which was wrong. Separation is based on the hind margin of the wing patch, either smooth or ragged. But that character varies within populations of *ancilla,* especially hybrid ones, and it turns out that most populations are hybrid. Another 'race', *orientalis*, was created, again based on the apical position of the male wing-patch (Selys 1887: 40). Androchrome females were correctly said to be common, which is not the case in the morphologically similar *Calopteryx s. syriaca* where true androchromy has never been observed. A second species completely lacking androchrome females is *C. xanthostoma.* A third such species is *C. exul.* It has hyaline wings in both sexes, and it was described as a 'race', a term corresponding to the modern concept of subspecies. In fact, it is a distinct species (see below). The three West Mediterranean endemics thus share an absence of androchromy.

The next major contributor to *Calopteryx* systematics was the Russian worker A.N. Bartenev (1912), who extended the range of observations to the east but published in journals not widely available in the west, except for a summary of his 1912 publication (Bartenev 1930). He too was confused by the variability of the wing patches, from fully absent to covering almost the entire wing surface. His solution avoided trinomial nomenclature but came at a price: Selys's »*races*« were raised to species rank. This was the case with *C. taurica*, *unicolor*, *orientalis*, and *syriaca*. *Calopteryx unicolor* Bartenev, 1912, is the post-emergence phase of *C. samarcandica.* It develops its diffuse wing patches only about a week after emergence. This constitutes a remarkable parallelism with *C. xanthosoma,* which also spends the first days of its adult life without any wing marking (Fig. 18).

Bartenev's system was adopted by Schmidt (1954) in his review of Iranian dragonflies. He was followed by B.F. Belyshev, who lived and worked at Novosibirsk and introduced one new synonym to *ancilla*, viz. *Calopteryx splendens johanseni* Belyshev, 1955, originally at the subspecies rank. The holotype was an androchrome female; the type locality is lake Gorkoye, a salt lake situated in a steppe *ca* 30 km north-west of Rubtsovsk and 270 km south-west of Barnaul. There are also rivers in the area, and probably the type specimen was a stray individual of fluvial origin. More specimens, including males, were later seen by Haritonov (1977), who raised the status to full species rank and widened its range to the foothills of the western Altai. The distinctive characters, however, were as in morph *ancilla*. In recent decades, morph *johanseni* has proved to be a common and widespread animal in Siberia.

In summary, one and a half centuries after Selys and Hagen attempted to use the male wing patch as well as the occurrence of androchrome females as the basis for a taxonomic framework, the taxonomy of the *splendens* group remained a tall edifice built on sand. Only in the south-east of the enormous range of the complex, much of which turns out to be in Siberia, were groups of populations encountered, sufficiently homogeneous to merit a species-group status different from *splendens*. They are, in the east, *orientalis, syriaca*, and *samarcandica,* and, in the south-west, *xanthostoma* and *exul.* It was felt by many that this was unsatisfactory, especially with respect to forms without or with a reduced wing patch. Efforts were made to remedy the situation by various approaches, but no hypothesis testing could be done until the advent of DNA-based methods.

The ground-breaking paper on the phylogeny of the Calopterygidae was provided by MISOF *et al.* (2000). It compared the sequences of the nuclear rRNA gene in *Calopteryx* species from Europe and some related species and genera from South Asia. The spacers ITS1 and ITS2 are stretches of DNA not translated into protein and hypervariable. Another paper, looking into the phylogeny of the entire order, based on calopterygoid damselflies 18S operon, was published by DUMONT *et al.* (2005). At the other extreme, several papers attempt to unravel the circumspecific phylogeny of *Calopteryx splendens sensu lato* (DUMONT *et al.* 2007), often in combination with a fragment of the mitochondrial cytochrome c oxidase subunit 1 (COI) (GUAN *et al*. 2012). In dragonflies, this turned out to be an alternative for ITS (*e.g.*, GUAN *et al*. 2013). It gained popularity, in recent years, especially in so-called barcoding studies, aimed at identifying species and in phylogenetic work (RATNASINGHAM & HEBERT 2013). Still another approach is the Amplified Fragment Length Polymorphism (AFLP) that has been especially popular in botany. It was used in *Calopteryx* by SADEGHI *et al*. (2010). It tests variation in hundreds of anonymous loci. The complete genome of *C. splendens* was sequenced in 2017 (IOANNIDIS *et al.* 2017). This opens up new possibilities for phylogenetic work.

Calopteryx splendens splendens and its relatives

Except for *splendens* itself, around 25 names (for *samarcadica* two, and for *xanthostoma* one synonym) – excluding four old synonymous names given for *C. s. splendens* in 1785–1839 (Hämäläinen & Fliedner 2022: 15) – have been proposed as subspecies or relatives of *splendens* and named, using unreliable morphological traits such as shape of post-ocular tubercles, body size, and selected wing venation traits. Here, all except two are rejected as being too weakly supported. *Calopteryx splendens orientalis* and *C. splendens syriaca* from the southern Caspian shore and the Jordan valley are accepted with rather weak support and a difference in the female hind wings. The subspecies status of *C. splendens hyalina* and *C. splendens waterstoni* is rejected because it is neither supported by morphology nor by DNA. Three confirmed species (*C. exul*, *C. samarcandica*, *C. xanthostoma*) earned distinct species status from analysis of the barcoding fragment of the COI gene and ITS1 and/or ITS2 of the rRNA operon, combined with morphology. We consider as *C. s. splendens* all populations not included in the above, including *hyalina*/*waterstoni* and *ancilla*/*intermedia*, despite their enormous variability.

What is _Calopteryx splendens splendens_?

We suggest that *njuja* is a morph, the northernmost Asian population of *C. s. splendens* currently known, genetically connected to the populations of the mountains of Crimea, the Danube headwaters, the British Isles, northwestern Iran, and Denmark. Furthermore, morph *waterstoni* and its hybrids in Anatolia also belong here and were part of a migration that began less than 20 000 years ago, somewhere in the Pontocaspian basin. This animal was a little more resistant to cold than another form that lived in the same area though unconnected to the first, and therefore had to wait until temperatures became high enough to allow it to expand its range. It is therefore no coincidence that the relicts of the first wave tend to be found at the northern fringe of the *splendens* range. We also accept that, once it started expanding, the second wave was stronger than the first and absorbed *waterstoni* by hybridization. After few generations, all that was left of the original *waterstoni* were some phenotypes with a suggestion of a wing band. But where conditions were such that *waterstoni* became isolated at the foot of a steep mountain range, preferably facing the sea, a high mountain, or a combination of these factors, it could survive to date.

Taxonomic conclusions

The latest published checklist of the available genus- and species-group names of the extant Calopterygidae taxa of the world was published by Hämäläinen & Fliedner (2022: 13–23). No less than a total of 35 available species-group names were listed in the *C. splendens*-group in the sense used in our paper. Over half of them were considered synonyms.

We arrive at the conclusion that, including the nominate taxon, only three subspecies in *Calopteryx splendens* can be maintained. However different some populations may look; they are still *C. splendens splendens*. Resolving patterns of evolution in the *C. splendens*-group is underway, but we are only at the beginning. To distinguish variants, a non-taxonomic term may be used. In the text we used the term 'morph', but other terms would be equally valid. We present below a list of valid names and synonyms in the *C. splendens*-group, both subspecies and species, as adopted in the present paper.

Valid names in the *Calopteryx splendens*-group

Calopteryx exul Selys, 1853
Calopteryx samarcandica Bartenev, 1912
  [including synonyms *C. maracandica* Bartenev, 1913, and *C. unicolor* Bartenev, 1912]
Calopteryx splendens splendens (Harris, 1780)
Calopteryx splendens orientalis Selys, 1887
  [including synonyms *C. orientalis risi* Schmidt, 1954, *C. splendens shachrudicus* Bartenev, 1916, and *C. transcaspica* Bartenev, 1912]
Calopteryx splendens syriaca Rambur, 1842
Calopteryx xanthostoma (Charpentier, 1825)
  [including synonym *Agrion splendens pfeifferi* Götz, 1923]

New synonyms of *Calopteryx splendens splendens*

Calopteryx splendens amasina Bartenev, 1912, syn. nov.
Calopteryx splendens ancilla Hagen in Selys, 1853, syn. nov.
Calopteryx splendens balcanica Fudakowski, 1930, syn. nov.

Calopteryx splendens caprai Conci in Conci & Nielsen, 1956, syn. nov.
[earlier ranked as synonym of *C. splendens ancilla*]
Calopteryx splendens cartvelica Bartenev, 1930, syn. nov.
[earlier ranked as synonym of *C. splendens intermedia*]
Calopteryx splendens ciscaucasica Bartenev, 1925, syn. nov.
[earlier ranked as synonym of *C. splendens mingrelica*]
Calopteryx splendens cretensis Pongracz, 1911, syn. nov.
[earlier ranked as synonym of *C. splendens ancilla*]
Calopteryx splendens erevanense (Akramowski, 1948), syn. nov.
[earlier ranked as synonym of *C. splendens tschaldirica*]
Calopteryx splendens hyalina Martin, 1909, syn. nov.
Calopteryx splendens intermedia Selys, 1887, syn. nov.
[earlier, *C. intermedia cecilia* Bartenev, 1912, *C. intermedia persica* Bartenev, 1912, and *C. splendens cartvelica* Bartenev, 1930, have been synonymised with *C. splendens intermedia*]
Calopteryx splendens johanseni Belyshev, 1955, syn. nov.
[earlier ranked as synonym of *C. splendens ancilla*]
Calopteryx splendens mingrelica Selys, 1869, syn. nov.
Calopteryx splendens njuja Kosterin & Sivtseva, 2009, syn. nov.
Calopteryx splendens taurica Selys, 1853, syn. nov.
Calopteryx splendens tschaldirica Bartenev, 1909, syn. nov.
Calopteryx splendens tuempeli Scholz, 1908, syn. nov.
[earlier ranked as synonym of *C. splendens ancilla*]
Calopteryx splendens waterstoni Schneider, 1984, syn. nov

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Appendix I. Genetic distance, estimated by using the Kimura 2-parameter model (KIMURA 1980), between different species, subspecies, and morphs of *Calopteryx*. The number of base substitutions per site from averaging over all sequence pairs between groups are shown.

	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
C. splendens caprai	0.000																						
C. splendens intermedia	0.997	0.000																					
C. splendens balcanica	0.000	0.976	0.000																				
C. splendens ancilla	0.327	1.229	0.245	0.000																			
C. splendens johanseni	0.000	0.997	0.000	0.327	0.000																		
C. splendens taurica	0.000	0.997	0.000	0.327	0.000	0.000																	
C. splendens waterstoni	1.147	1.991	1.068	0.490	1.147	1.147	0.000																
C. splendens cretensis	0.000	0.916	0.000	0.327	0.000	0.000	0.984	0.000															
C. splendens hyalina	0.204	0.939	0.205	0.532	0.204	0.204	1.273	0.122	0.000														
C. xanthostoma	0.016	1.008	0.016	0.344	0.016	0.016	1.165	0.016	0.220	0.000													
C. splendens splendens	0.163	1.163	0.164	0.491	0.163	0.163	1.313	0.163	0.367	0.180	0.000												
C. splendens orientalis	0.621	1.547	0.608	0.953	0.621	0.621	1.778	0.623	0.827	0.632	0.786	0.000											
C. splendens tschaldirica	0.339	1.242	0.339	0.682	0.339	0.339	1.538	0.339	0.552	0.356	0.475	0.373	0.000										
C. splendens syriaca	0.653	1.596	0.657	0.986	0.653	0.653	1.813	0.655	0.860	0.671	0.818	1.281	1.022	0.000									
C. samarcandica	2.657	2.706	2.593	2.493	2.657	2.657	3.136	2.505	2.605	2.656	2.820	3.001	2.718	3.131	0.000								
C. exul	7.251	7.558	7.195	7.100	7.251	7.251	7.811	7.089	7.031	7.280	7.436	7.795	7.555	7.995	8.190	0.000							
C. virgo meridionalis	10.373	10.392	10.330	10.617	10.373	10.373	11.354	10.212	10.146	10.352	10.371	10.942	10.225	10.785	10.616	12.311	0.000						
C. virgo meridionalis	10.568	10.586	10.526	10.814	10.568	10.568	11.552	10.406	10.340	10.547	10.566	11.138	10.426	10.982	10.813	12.110	0.163	0.000					
C. virgo virgo	10.752	10.771	10.710	10.998	10.752	10.752	11.738	10.590	10.523	10.731	10.750	11.283	10.617	11.166	10.965	12.700	0.489	0.653	0.000				
C. virgo padana	10.568	10.586	10.526	10.814	10.568	10.568	11.552	10.406	10.340	10.547	10.566	11.138	10.426	10.982	10.813	12.512	0.326	0.489	0.163	0.000			
C. haemorrhoidalis	15.216	15.579	15.198	15.076	15.216	15.216	15.424	15.055	15.393	15.240	15.380	15.780	15.474	15.663	16.331	16.465	16.713	16.928	16.911	16.928	0.000		
C. aequabilis	18.350	18.348	18.261	18.644	18.350	18.350	19.662	18.290	18.200	18.318	18.480	18.899	18.119	18.613	18.876	18.884	19.743	19.971	19.721	19.515	19.228	0.000	
C. amata	18.020	18.017	17.909	18.310	18.020	18.020	19.317	17.960	17.871	17.988	18.149	18.563	17.778	18.279	18.655	18.770	19.521	19.748	19.500	19.295	18.687	1.320	0.000

Appendix II. Information on Calopterygidae specimens sequenced for this study. For some species COI was sequenced new, but ITS (**bold**) was sequenced earlier and was already published on GenBank (Benson *et al*. 2008). Two-letter country codes according to ISO 3166-1 alpha-2 codes. HJD, MH, MP, SNB, TS – initials of authors; CC – Country code.

Specimen	CC	Region	Collector, year	PCR-COI	PCR-ITS	GenBank-COI	GenBank-ITS
Calopteryx Leach in Brewster, 1815							
C. aequabilis Say, 1840	US	Wisconsin	S.W. Dunkle, 1996	15351	–	PV069669	–
C. amata Hagen, 1889	CA	New Brunswick	S.W. Dunkle, 1996	15382	–	PV069668	–
C. cornelia Selys, 1853	JP	Odawara	Coll. HJD	13088	–	PV069673	–
C. exul Selys, 1853	MA	Région Béni Mellal-Khénifra, Khénifra	HJD, 1996	9274	12138	PV069660	PV076660
C. exul	MA	Région Fès-Meknès, Ifrane	HJD, 1996	12078	–	PV069661	**AJ308346**
C. haemorrhoidalis (Vander Linden, 1825)	MA	Région Béni Mellal-Khénifra, Khénifra	HJD, 1996	10345	–	PV069666	–
C. haemorrhoidalis	MA	Région Fès-Meknès, Ifrane	HJD, 1996	12079	–	PV069667	–
C. s. ancilla Selys, 1853	PL	Western Poland	HJD, 2002	11882	12119	PV069640	PV076703
C. s. ancilla	AZ	Baku	Coll. HJD	–	12129	–	PV076680
C. s. balcanica Fudakowski, 1930	GR	Peloponnese, Kalavrita	TS, 2001	10799	12151	PV069633	PV076696
C. s. balcanica	GR	Euboea, Kirinthos	W. Lopau, 1997	11880	12173	PV069654	PV076667
C. s. balcanica	HR	Dalmatia, Puljane, Miljacka waterfall	Coll. HJD	12116	12106	PV069617	PV076673
C. s. caprai Conci in Conci & Nielsen, 1956	IT	Campania, Vallo di Diano	M. Pavesi, 1996	15992	12124	PV069630	PV076656
C. s. caprai	IT	Campania, Vallo di Diano	M. Pavesi, 1996	12052	–	PV069616	**AJ308370**
C. s. caprai	IT	Frosinone Prov., Pontecorvo	A. Cordero, 1999	12053	–	PV069625	**AJ308371**

Specimen	**CC**	**Region**	**Collector, year**	**PCR-COI**	**PCR-ITS**	**GenBank-COI**	**GenBank-ITS**
C. s. caprai	IT	Lombardy, Tromello	M. Pavesi, 1996	15994	12126	PV069631	PV076695
C. s. cretensis Pongracz, 1911	GR	Crete, Mixórrouma	M. Wasscher, 1999	12069	–	PV069612	**AJ308364**
C. s. hyaline type	GE	Kvemo Kartli Region, Paravami creek N Orlowka	TS, 2015	9331	12139	PV069607	PV076681
C. s. hyaline type	GE	Kvemo Kartli Region, Paravami creek N Orlowka	TS, 2015	9332	12140	PV069608	PV076675
C. s. hyalina Martin, 1909	SY	Medhala, Wadi Al Kabir	HJD, 2010	9378	12191	PV069609	PV076688
C. s. hyalina	SY	Baniyas, R. Nahr as-Sinn	HJD, 2010	9424	–	PV069611	–
C. s. intermedia Selys, 1887	TR	*Muğla* Prov., Köyceğiz gölü	M. Pavesi, 1997	10262	12128	PV069610	PV076678
C. s. intermedia/ orientalis	AZ	Balakan Distr., Çiçibinə	N. Snegovaya, 2014	11766	12157	PV069641	PV076694
C. s. intermedia/ orientalis	AZ	Qabala Distr., Vandam	N. Snegovaya, 2016	11485	12156	PV069636	PV076697
C. s. intermedia	IR	Kermanshah Prov., Qasr-e Shirin, R. Alvand	TS, 2017	10897	12152	PV069649	PV076693
C. s. intermedia	IR	Ardabil Prov., E Sarāb	TS, 2016	–	12146	–	PV076683
C. s. johanseni Belyshev, 1955	RU	Altai, Krasnogoskoje	HJD, 1999	11869	12123	PV069618	PV076677
C. orientalis/ intermedia	IR	Khorasan-e-Razavi Prov., N Zavadar	TS, 2016	10493	12144	PV069639	PV076690
C. s. orientalis/ intermedia	IR	Khorasan-e-Razavi Prov., W Zavadar	TS, 2016	11043	12148	PV069642	PV076692
C. s. orientalis Selys, 1887	AZ	Lenkoran, Azfilial, NW Moğonojoba	N. Snegovaya, 2017	11484	12155	PV069636	PV076697
C. s. orientalis	IR	Khorasan-e-Shomali Prov., R. Atrak NW Bodschnurd	TS, 2016	10706	12147	PV069650	PV076691

Specimen	CC	Region	Collector, year	PCR-COI	PCR-ITS	GenBank-COI	GenBank-ITS
C. s. orientalis	IR	Khorasan-e-Shomali Prov., SE Bodschnurd	TS, 2016	10208	12143	PV069637	PV076689
C. s. splendens (Harris, 1780)	FI	Satakunta Prov., Ahlainen	MH, 1999	10263	12127	PV069632	PV076674
C. s. splendens	FR	Bretagne	HJD, 1996	12046	–	PV069626	–
C. s. splendens	FR	Dépt Alpes-de-Haute-Provence, Manosque, R. Durance	MP, 1999	12047	–	PV069615	**AJ308368**
C. s. splendens	RU	West Siberia, Tomsk	local collector, 2015; Coll. TS	–	12150	–	PV076684
C. s. splendens	IE	Cork	A. Bond, date unclear	12045	–	PV069645	**AJ308365**
C. s. splendens	SI	R. Ljubljanica, Ljubljana	A. Brancelj, 1996	12048	–	PV069619	**X98502**
C. s. syriaca Rambur, 1842	SY	Wadi Al Kabir near Medhala	HJD, 2010	10206	12142	PV069646	PV076682
C. s. syriaca	IL	R. Jordan W Had Nes	TS, 2013	10733	12149	PV069647	PV076679
C. s. taurica Selys, 1853	DK	Jutland, Skanderborg munic., Ry	O.F. Nielsen, 1996	11877	12122	PV069621	PV076704
C. s. tschaldirica Bartenev, 1909	IR	Azarbãyejãn-e-Garbi Prov., R. Aras NW Poldasht	TS, 2017	10756	12153	PV069638	PV076676
C. s. waterstoni Schneider, 1984	TR	Yanbolu Çayı E Trabzon	TS, 2012	11347	12154	PV069648	PV076686
C. samarcandica Bartenev, 1912	TJ	Khatlon Prov., Kodara	SNB, 2019	14669	14684	PV069654	PV076667
C. samarcandica	TJ	Khatlon Prov., Kodara	SNB, 2019	14672	14687	PV069656	PV076671
C. samarcandica	TJ	Khatlon Prov., Kodara	SNB, 2019	14747	14681	PV069657	PV076670
C. samarcandica	TJ	Khatlon Prov., Kodara	SNB, 2019	14748	14683	PV069658	PV076669
C. samarcandica	TJ	Khatlon Prov., Kodara	SNB, 2019	14750	14686	PV069655	PV076668

Specimen	CC	Region	Collector, year	PCR-COI	PCR-ITS	GenBank-COI	GenBank-ITS
C. samarcandica	TJ	Khatlon Prov., Kodara	SNB, 2019	14809	14685	PV069653	PV076666
C. samarcandica	TJ	Khatlon Prov., Kodara	SNB, 2019	14667	14682	PV069659	PV076672
C. samarcandica	TM	Koytendag Region, Koyten	SNB, 1989; coll. HJD	9595	–	PV069651	–
C. samarcandica	KZ	Jetisu Region, Kalinino	SNB, 2018	12098	12105	PV069629	PV076661
C. samarcandica	KZ	Jetisu Region, Kalinino	SNB, 2018	12104	12109	PV069627	PV076662
C. samarcandica	KG	Sary-Chelek Reserve, Arkit	SNB, 1985; coll. HJD	11847	12159	PV069652	PV076665
C. samarcandica	KG	Sary-Chelek Reserve, Arkit	SNB, 1985; coll. HJD	12226	12275	PV069644	PV076663
C. samarcandica	KG	Sary-Chelek Reserve, Arkit	SNB, 1985; coll. HJD	12228	12276	PV069643	PV076664
C. virgo (Linnaeus, 1758)	FR	Dépt Aisne, Laon	HJD, 1999	12110	–	PV069664	*AJ308359*
C. virgo feminalis Bartenev, 1910	AZ	Balakan Distr., Çiçibinə	N. Snegovaya, 2014	–	12158	–	PV076698
C. virgo padana Conci in Conci & Nielsen, 1956	SI	R. Ljubljanica, Ljubljana	A. Brancelj, date unclear	12113	–	PV069665	**AJ308356**
C. virgo meridionalis Selys, 1873	FR	Dépt Lot, Cabrerets	HJD, 1996	12080	–	PV069662	**AJ308358**
C. virgo meridionalis	ES	León, Bierzo	K. Van Damme, 1999	12111	–	PV069663	**AJ308357**
C. xanthostoma (Charpentier, 1825)	FR	Fleuve l'Argens, Chateauvert	MP, 1999	11448	12120	PV069628	PV076700
C. xanthostoma	FR	Dépt Lot, Cabrerets	MP, 1996	12055	–	PV069623	**AJ308352**
C. xanthostoma	IT	Liguria	M. Pavesi, 1996	12071	–	PV069634	**AJ308353**

Specimen	**CC**	**Region**	**Collector, year**	**PCR-COI**	**PCR-ITS**	**GenBank-COI**	**GenBank-ITS**
C. xanthostoma	IT	Toscana, Siena	M. Pavesi, 1996	11808	12130	PV069614	PV076657
C. xanthostoma	IT	Liguria, Albenga	M. Pavesi, 1996	–	12133	–	PV076659
C. xanthostoma	ES	Galicia, Pontevedra	A. Cordero, 1999	12058	–	PV069624	**AJ308355**
C. xanthostoma	ES	Valencia	C. Bonet, 1996	11451	12132	PV069613	PV076658
C. xanthostoma	ES	Valencia	C. Bonet, 1996	12056	–	PV069620	**AJ308351**
Atrocalopteryx Dumont, Vanfleteren, De Jonckheere & Weekers, 2005							
A. melli (Ris, 1912)	CN	Nanskunshan	Coll. HJD	9376	–	PV069670	PV076655
A. atrocyana (Fraser, 1935)	CN	Tian Men Gou	Coll. HJD	9385	–	PV069672	–
A. coomani (Fraser, 1935)	VN	Da Nang	Coll. HJD	11872	–	PV069671	–
Iridictyon Needham & Fisher, 1940							
I. myersi Needham & Fisher, 1940	VE	Sierra de Lema	HJD, 2001	15989	–	PV069606	*AJ459227*