

Morphological and molecular evidence supports the species status of the Italian endemic *Coenagrion castellani* Roberts, 1948 (Coenagrionidae)

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All relevant data are within the paper and its [Supporting Information files](#).

Abstract. *Coenagrion castellani* Roberts, 1948 was described from Italy as a distinct species almost 75 years ago but has generally not been recognised or was treated as a subspecies of *C. mercuriale* (Charpentier, 1840). Populations south of the Alps were recently shown to be completely isolated genetically from those in North Africa and elsewhere in Europe. As markings and male appendages also allow for easy separation in the field, *C. castellani* is best treated as a good species, the 146th odonate species known from Europe and the second one that is endemic to Italy. Its identification and occurrence are reviewed. North African populations are distinct genetically too, but not in morphology. Whether these should be treated as a distinct taxon, e.g. as the subspecies *C. mercuriale hermeticum* (Selys, 1872), requires further research.

Key words. Odonata, dragonfly, damselfly, Zygoptera, Italy, phylogeography, taxonomy.

Introduction

Following its discovery in eastern England, James Ernest Helme Roberts (1948) requested Italian specimens of the Dainty Bluet *Coenagrion scitulum* (Rambur, 1842) from the Italian entomologist Cesare Conci for comparison. The material received included a species nearer the Mercury (or Southern) Bluet *C. mercuriale* (Charpentier, 1840), which he found distinct enough to describe as new. Reviewing the description, Conci (1949) noted that Roberts's female belonged to the Mediterranean Bluet *C. caerulescens* (Fonscolombe, 1838). While he found no characters to separate Italian females from other *mercuriale* females, he agreed that Italian males differed, but suggested treating Roberts's taxon as a subspecies. He emended the name (which honours the collector Omero Castellani) to *castellanii*, but this was unjustified (ICZN Art. 33.4).

The work by Roberts (1948) and Conci (1949) was succinct, with little on differentiating *castellani* from *mercuriale*. Roberts's mix-up with *C. caerulescens*, moreover, undermined his credibility. Also, Ben Azzouz et al. (1989a) reported *C. castellani* from Morocco, even alleging to describe its larva (Ben Azzouz et al., 1989b). All her material, however, probably pertained to *C. caerulescens* or *C. scitulum* or possibly both (Jacquemin & Boudot, 1990). Such work may well have facilitated

the view that the Italian populations fell within the variation of *C. mercuriale* across its ecologically and geographically wide range from the arid Maghreb to the damp heaths of Great Britain.

Among the popular literature, for example, Conci & Nielsen (1956), Aguesse (1968), Geijskes & van Tol (1982), D'Aguilar et al. (1985), Askew (1988), and Bos & Wasscher (1997) only mentioned the Italian taxon or briefly diagnosed it as a subspecies. Robert (1958), Bellmann (1987), Nüss & Wendler (1991; updated as Lehmann et al., 2015), Galliani et al. (2017), Wildermuth & Martens (2018), and Smallshire & Swash (2020) did not include it at all. Boudot et al. (2009) and Boudot & Kalkman (2015) stated that no subspecies of *C. mercuriale* can be recognised. Dijkstra & Lewington (2006) mentioned the possibility of an Italian subspecies without stating distinguishing characters, but removed that remark from a later edition (Dijkstra et al., 2020).

Galimberti et al. (2021) compared sequences of the mitochondrial 5'-end COI gene region (the standard 'DNA barcode', 658 bp) from seven Italian specimens with 30 available in GenBank, as well as 145 samples from other parts of Europe and North Africa, almost all originating from the PhD work of Sónia Ferreira (Ferreira et al., 2014; unpublished). Three haplogroups that did not overlap in their geographic range were found. Each of these is associated with a published name: (1) *castellani* Roberts, 1948 (type locality: Rome, Italy): restricted to the Italian peninsula, (2) *mercuriale* Charpentier, 1840 (Lüneburg, Germany): Spain, Portugal, France, Great Britain, Germany, and Switzerland; and (3) *hermeticum* Selys, 1872 (Algeria): Morocco, Tunisia, and Algeria. Hundreds of sequences from another mitochondrial locus (16s rRNA), as well as nine nuclear loci, are also available in GenBank (S. Ferreira, unpublished), and in almost all cases these showed unique haplotypes in Italy too (Galimberti et al., 2021).

These molecular results prompted us to (1) analyse the available genetic data in a geographic context; (2) review the morphological identification of the Italian populations, and (3) re-evaluate the taxonomy, distribution and status of the latter.

Methods and material

Genetics and phylogeography

To assess the genetic divergence patterns within the entire range of *C. mercuriale* (sensu lato) we assembled multiple datasets including the nucleotide sequences obtained by Ferreira et al. (2014) and Galimberti et al. (2021) and all those available from GenBank. Five alignments, two from the mitochondrial (COI and 16s rRNA) and three from the nuclear genome (MLC, PRMT, and AgT) were selected for phylogeographic analysis, since these represented most individuals and countries sampled, and were also the most variable; see Ferreira et al. (2014) and Galimberti et al. (2021) for details and Supplementary table 1 for the selected sequences.

For each dataset, the nucleotide sequences were aligned and unphased as described by Ferreira et al. (2014) and the two mtDNA fragments were concatenated and treated as one locus. Each alignment was collapsed in haplotypes with DnaSP v6 (Rozas et al., 2017) and the unrooted minimum spanning networks were built using the median-joining algorithm (Bandelt et al., 1999) with default settings in PopART (<http://popart.otago.ac.nz>; Leigh & Bryant, 2015) (Fig. 1).

Morphology and distribution

Individuals associated with the Italian haplotype group were compared with those of the other groups, with dimensions of hindwings, appendages, and markings measured from collected males and (for the latter feature) field photographs. As the type localities of the relevant taxa are clear, and Roberts's (1948) description was sufficient, the study of type material was deemed unnecessary. Larval characters were not considered.

The diagnosis based on these comparisons (see below and Figs 2–3) was used to verify photographic records on citizen science platforms such as Ornitho.it, iNaturalist.org and Observation.org, as well as unpublished data from colleagues. The database of the Italian odonatological association Odonata.it, which includes historic collections, literature, and new unpublished records, was reviewed to complete an up-to-date overview of the distribution (Fig. 4) and flight period in Italy.

The following specimens were examined morphologically. The acronym RMNH refers to the collection of Naturalis Biodiversity Center, formerly Rijksmuseum van Natuurlijke Historie (National Museum of Natural History of The Netherlands), Leiden. FRANCE: 2 ♂♂, 2 ♀♀, Dordogne, ca 2 km south-southeast of St Cyprien, 21 June 1986, leg. J. van Tol & S. Kofman, coll. RMNH; 1 ♀, Dordogne, Carsac-Aillac, 5 June 1975, leg. D.C. Geijskes, coll. RMNH; 1 ♂, Hérault, Source du Lez, 29 May 1979, leg. G.S. Vick, coll. RMNH (ex coll. M. Härmäläinen); 3 ♂♂, Lot, Borrèze valley, Salignac-Souillac road, 23 June 1965, leg. M.A. Lieftinck, coll. RMNH; 1 ♀, Lot, Rostassac, 20 June 1965, leg. M.A. Lieftinck, coll. RMNH. GERMANY: 4 ♂♂, Bavaria, Steingarden-Trauchgau, Biberschwöllersee, 770 m, 10 July 1974, leg. M.A. Lieftinck, coll. RMNH. ITALY: 1 ♂, Basilicata, Rotondella, 22 June 2011, leg. & coll. F. Landi; 2 ♂♂, Basilicata, Senise, 25 June 2011, leg. & coll. F. Landi; 1 ♂, Calabria, Spilinga, 15 June 1995, leg. C. D'Antonio, coll. F. Landi; 2 ♂♂, Campania, Gallo Matese, 6 July 1994, leg. C. D'Antonio, coll. F. Landi; 1 ♂, Lazio, Antico Lavatoio Sermoneta, 20 April 2019, leg. S. Ramellini, coll. G. Assandri; 1 ♀, Lazio, Antico Lavatoio Sermoneta, 7 June 2020, leg. S. Ramellini, coll. G. Assandri; 4 ♂♂, 1 ♀, Lazio, Cassino, Frosinone, 24 May 2021, leg. & coll. A. Corso; 2 ♂♂, Marche, Camerano, 28 June 2008, leg. & coll. F. Landi; 1 ♂, Marche, Macerata, 16 August 1979, leg. & coll. F. Landi; 1 ♂, Marche, Macerata, 27 August 1980, leg. & coll. F. Landi; 2 ♂♂, Marche, Montecosaro, 23 May 2010, leg. & coll. F. Landi; 1 ♂, 1 ♀, Piemonte,

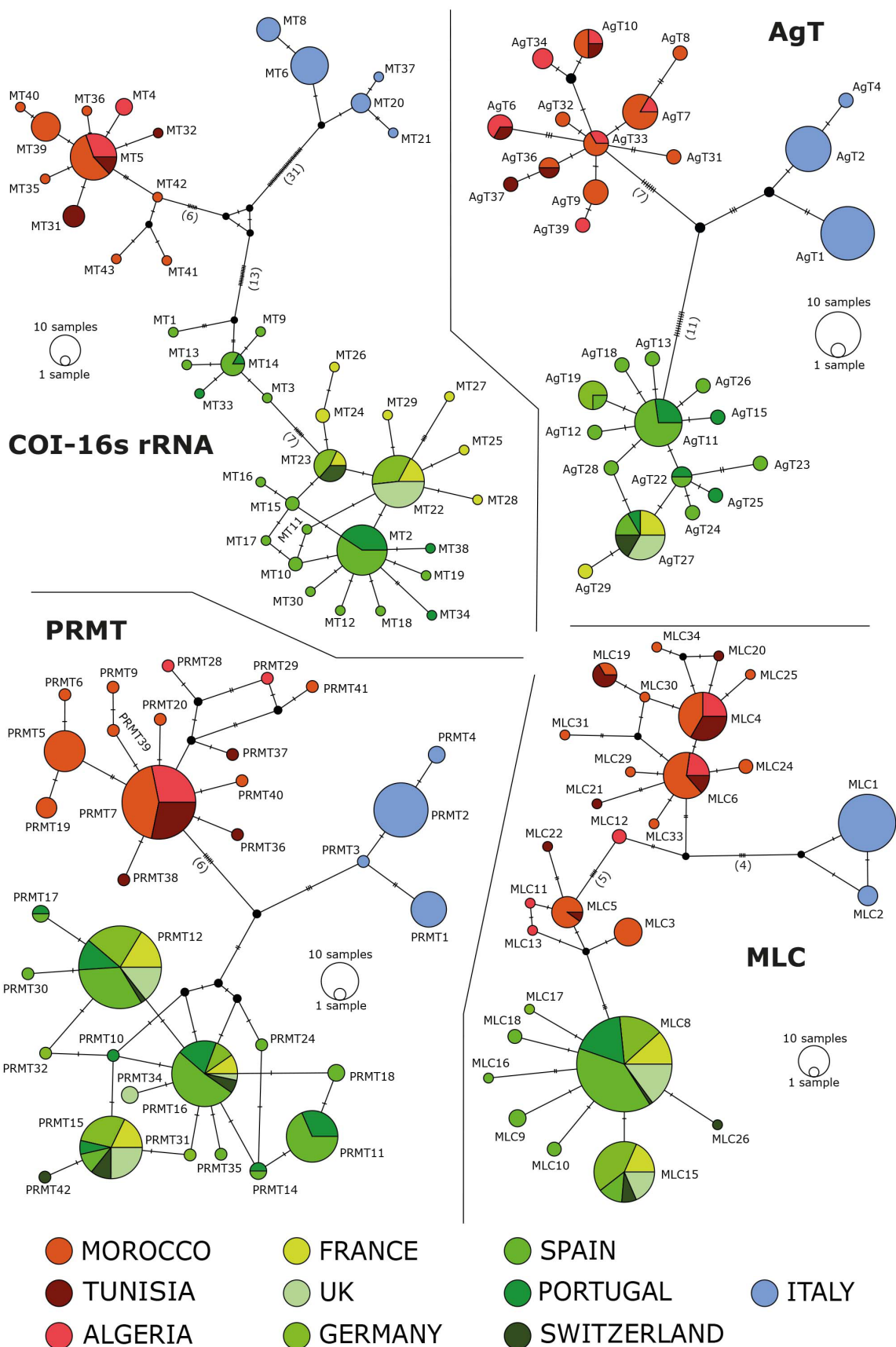


Figure 1. Median-joining network of *Coenagrion mercuriale* s.l. haplotypes for the concatenated cytochrome c oxidase I (COI) + 16S ribosomal RNA gene (16s rRNA) (1100 bp), acetylglucosaminyl-transferase (AgT, 612 bp), arginine methyltransferase (PRMT, 705 bp) and myosin light chain (MLC, 236 bp) alignments (see Supplementary Table 1 for haplotypes and country subdivision). Each circle represents a haplotype, and circle size is proportional to haplotype frequency. Colours indicate different sampling countries, with red tones referring to *hermeticum*, green tones to *mercuriale*, and blue tones to *castellani*. Small black dots represent median vectors (i.e., possibly unsampled haplotypes), while dashes represent substitutions (also indicated within brackets when >3).

Fosso dint. Stagni S. Sebastiano, Fossano, 30 June 2019, leg. A. Galimberti, coll. G. Assandri; 2 ♂♂, 1 ♀, Roma, Montebello, 2 May 1948, leg. Bisieto F. Donate, coll. RMNH (ex coll. C. Consiglio). MOROCCO: 1 ♂, High Atlas, Asni-Imlil, 1150–1800 m, 6 June 1966, leg. M.A. Lieftinck, coll. RMNH; 1 ♂, High Atlas, Ourika valley, east of Asni, 1300 m, 4 June 1973, leg. M.A. Lieftinck, coll. RMNH; 3 ♂♂, Middle Atlas, 5 km south of Timahdite, 1900 m, 27 May 1966, leg. M.A. Lieftinck, coll. RMNH. PORTUGAL: 1 ♂, Rio Maior, 8 July 1963, leg. unknown, coll. RMNH (ex coll. Leclercq-Sombloux). SPAIN: 1 ♀, Caceres, Abadia, 5 May 1960, leg. Exc. RMNH, coll. RMNH; 3 ♂♂, León, Río Cea, bridge at Villaverde de Arcayos, 14 July 1984, leg. J. Belle, coll. RMNH; 1 ♂, Teruel, 25–30 km southwest of Alcaniz, 350 m, 10 July 1972, leg. J.P. Duffels, coll. RMNH; 1 ♂, 1 ♀, San Andrés, 50 km north of Soria, 30 June 1969, leg. H. Overbeek, coll. RMNH. UNITED KINGDOM: 1 ♀, Hampshire, Crockford Bog, New Forest, 1 July 1978, leg. G.S. Vick, coll. RMNH; 2 ♂♂, Hampshire, Crockford Bog, New Forest, 15 July 1978, leg. G.S. Vick, coll. RMNH.

Results and discussion

Genetics

The multi-approach species delimitation using COI sequences clearly showed the divergence of the Italian specimens from other European populations, as well as from the North African ones. Indeed, the haplotype networks presented here show that the Italian populations are well-separated from the two other groups at both the two mitochondrial and three nuclear loci (Fig. 1; Supplementary material Appendix S6). Galimberti et al. (2021) provided the genetic distance values between the three groups of populations in their Supplementary material Appendix S7: the three showed consistent but similar values of uncorrected genetic *p*-distances at the nuclear loci, while the Italian populations were comparatively distinct for the mitochondrial loci, with the highest values relative to the populations from the rest of Europe.

Morphological identification

Adult males assigned to *castellani* are consistent in appearance across the Italian peninsula and thus seem less variable than *mercuriale*, presumably on account of their more restricted range and ecology (Fig. 2). The marking on S2, for example, is rather constant in shape. Nonetheless, occasional males may have one or two markings recalling the other taxon: some *mercuriale* have a mark on S2 typical of *castellani*, for example, just as the latter can have markings on S3 more like *mercuriale*. Especially in North Africa, *mercuriale* can be dark and recall *castellani*. Extremely dark individuals may thus in theory have reduced pale markings on the head (as described for *castellani* below), although no examples of this have been seen. The genital ligula

(functional penis) of the only Italian male in which this was extracted did not differ from those of a German and a Moroccan male.

General appearance, build, and size (HW 17.0–19.5 mm, *n* = 14) are similar to *mercuriale* (16.0–19.5 mm, *n* = 21) but black markings are configured differently, as seen best on S2–6: while the markings extend similarly far along the abdominal dorsum, they are more extensive laterally, thus appearing thicker on average (Figs 2a–b). For example, when measured along the dorsum, the black markings on S3 cover 55–65% of the segment's length in both taxa, while their dorsal extent is similar on S5 too: 47–62% in *castellani*, 46–55% in *mercuriale*. There is no overlap, however, when the markings on S5 are measured along their sides: in *mercuriale* they never extend beyond 61% of the segment's length, while in *castellani* they cover 64–86%, owing to the greater development of lateral spikes (see below).

Pale markings on the underside of the head become increasingly narrow as they extend backwards along the eye margin and end abruptly, thus leaving the back of the head black except for the postocular spots and the stripe between them. These pale areas widen and extend clearly towards the centre of the head in *mercuriale*. As a consequence, when viewed from behind and above (as most photos are taken), the head of *mercuriale* usually shows a second pair of spots, which are similarly wide as the postocular spots above them.

Black marking on S2 is rather massive and rectangular, like a stalked square bearing two short and wide-based horns. Compared with *mercuriale*, which most commonly shows a triangular hood carrying two curved and slender horns, the marking is laterally more filled out, with much-thickened horns fused broadly to the hood. The marking thus appears quite like the cat's head of *C. caerulea* or *C. scitulum*, but with shorter and wider ears (i.e. the horns).

Black marking on S3 tends to be broad and truncated towards the front, typically ending in three small and fairly equal points. The lateral points are often somewhat thicker than the central point, extending a fraction further forward. The marking's anterior end is usually rounded or pointed in *mercuriale*, without lateral points. If such points are present, the central point clearly reaches forward further.

Black markings on S4–6 have more developed forward-pointing lateral spikes: on S5, for example, they occupy about 25–40% of the marking's full length, while in *mercuriale* they rarely reach 20% and are often even absent.

Black markings on S9–10 more extensive, notably on S10 where blue colour extends upward on the segment sides to about the level of the paraprocts, but often to the level of the cerci in *mercuriale*.

Male appendages are similar in structure to those of *mercuriale* (Fig. 3). However, the cerci are slightly longer, typically being 75–90% as long as S10 in lateral view (average 82%, $n = 16$), versus 65–85% in *mercuriale* (average 76%, $n = 21$). By contrast, the paraprocts are a fraction shorter (55–70% and 63%, versus 65–77% and 70%). As a result, the cerci appear 20–40% longer than the paraprocts in *castellani*, thus projecting well beyond them in lateral view, but are not or at most 10% longer in *mercuriale*. This difference can be seen quite easily even in field photographs. The cerci also appear

more drawn out, with a noticeably longer hook at their tips in dorsal view.

Adult females associated with *castellani* and *mercuriale* males are similar, but few were available for comparison. Any difference in the hind border of the pronotum is unappreciable, although its central lobe may be a fraction broader in *castellani*. The distinction of the markings on the underside of the head also appears to apply to females, however. Furthermore, the black marking on S2 may differ subtly: in *castellani* this is broad and narrowed about equally gradually and



Figure 2. Males with typical markings of (a) *C. castellani*, and (b) *C. mercuriale*. Photo (a) by Alexandro Minicò (Fossano, Italy), (b) by Carlo Galliani (Camargue, France).

deeply at the very base of the segment and at its midpoint, while in *mercuriale* the black is narrower overall, narrows rather deeply and abruptly at midpoint, but often does not narrow at the segment base at all.

Available keys do not distinguish between *castellani* and *mercuriale* larvae and are, with the exception of Carchini (2016), probably based exclusively on material pertaining to *mercuriale* (e.g., Brochard et al., 2014; Conesa Garcia, 2021; Gerken & Sternberg, 1999; Heidemann & Seidenbusch, 2002). Further investigations must thus clarify whether identification by larval characters is possible.

Distribution and flight period

Records have been obtained from 135 UTM grid squares of 100 km² in Italy since 1877 (Fig. 4). Of these 135 squares, 57% were occupied only after 2000, 26% only between 1950 and 1999, 8% only before 1950, and 9% both before and after 2000. Squares are occupied in all regions of southern and central continental Italy, with fewer towards the north, reaching the northern edge of the Apennines in Emilia-Romagna (Fabbri, 2018).

There are just a few isolated populations further west, with only two records from Liguria: one from the Apennines near Genova (Capra, 1945) and a recently discovered population in eastern Liguria close to Tuscany that is almost contiguous with the central Italian populations (G. Bernazzani & S. Hardersen, pers. comm.). The north-western limit in Italy is reached in Piedmont, where populations were first reported in the late 1990s and about ten are currently known from the plain near Fossano and the western border of the hills of Monferrato near Asti (Boano et al., 2007; Sindaco et al., 2018). There are no records from Italy north of the Po River; old data assigned to *C. mercuriale* from southern Switzerland (see Boudot & Kalkman, 2015) are no longer considered valid (Hepenstrick et al., 2021).

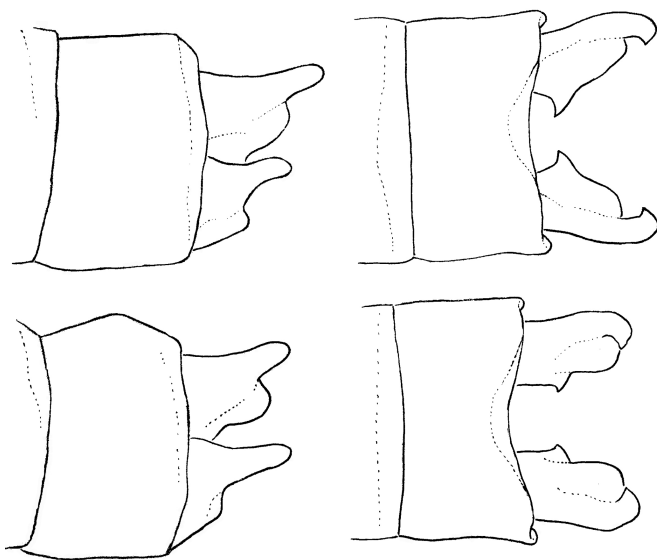


Figure 3. Male appendages (lateral view on left, dorsal view on right) of *C. castellani* (above), and *C. mercuriale* (below).

Records from Sardinia and Corsica are unconfirmed (Berquier, 2013), but Bucciarelli (1971, 1977) reported two localities in Sicily: his record from 1969 referred to *C. scitulum*, but Fiume Simeto at Ponte Bolo allegedly harboured a good population in 1973 and 1975 (Maurizio Pavesi, pers. comm.). The specimens have not been re-examined, while the habitat has disappeared. No further records from the island have been obtained since, despite sufficient research (A. Corso, pers. comm.).

Adults are on the wing from late March to the end of August, with only occasional records in September and October. Activity is concentrated in the second half of April, May, and June.

Evolution and biogeography

Our results show that, while populations within each of the three regional groups in this complex likely have genetic exchange, the groups themselves have long been separated (Fig. 1). The Italian populations' isolation from those in the rest of Europe, moreover, appears older than that of the North African ones. The data are unsuitable for molecular clock analyses, however. Swaegers et al. (2014) estimate that *mercuriale* and *castellani* diverged between 2.7 and 1.0 million years ago. This is before the separation of the Azure *C. puella* (Linnaeus, 1758) and Variable Bluets *C. pulchellum* (Vander Linden, 1825), which occur together widely across the Western Palaearctic, and even before the Prairie Bluet *C. angulatum* Walker, 1912 from North America diverged from its Eurasian sister-species, the Crescent Bluet *C. lunulatum* (Charpentier, 1840).

The Alps still appear to form a formidable barrier between the two taxa. While *castellani* might meet *mercuriale* in north-western Italy, adjacent France or Switzerland, the gap between them appears to be real. For example, *mercuriale* is quite common in the bordering French regions of Provence-Alpes-Côte d'Azur and Rhône-Alpes but becomes very rare towards the Alps (Deliry, 2008; Lamouille-Hébert, 2016; Papazian et al., 2017).

Taxonomic implications

Species delimitation is challenging because it requires the arbitrary selection of variable traits whose accuracy is often debated (Mayr, 1942). For odonates, Dijkstra & Lewington (2006) applied a simple point system to decide how to treat pairs of taxa in their field guide, arguing that species must be separable by their appearance and should not interbreed freely. For each pair, they determined whether distinguishing characters were absent (0 points), weakly defined such as in coloration or size (1 point), or discrete, like structural details or clear genetic divergence (2 points). Additionally, they gauged whether ranges graded into each other (0), were entirely separate so that possible intergradation could not be tested (1), or that these overlapped without intergrad-

ing or with a limited degree of hybridisation (2). Taxa for which the sum of these two criteria was three points or more were treated as distinct species, with questionable cases being given the benefit of the doubt.

Based on the evidence, *castellani* is distinct both morphologically and genetically, which amounts to two points. As yet there is no evidence of geographic overlap or genetic introgression, adding another point. By these criteria, *C. castellani* should be treated as a good species, as done in the recent Italian edition of the aforementioned guide (Dijkstra et al., 2021). Indeed, its status is more clearcut than that of the region's other endemic odonate, the Italian Goldenring *Cordulegaster trinacriae* Waterston, 1976. That species overlaps with the Common Goldenring *C. boltonii* (Donovan, 1807) in central Italy, with frequent hybridisation (Solano et al., 2018; Corso, 2019). With the addition of *C. castellani*, the number of Odonata species known in Europe

stands at 146 (Boudot & Kalkman, 2015; López-Estrada et al., 2020; Viganò et al., 2017).

Although the data show that North African populations form a genetically distinct lineage too (Fig. 1), morphological differences with typical *mercuriale* are unsubstantial (Jacquemin & Boudot, 1990, 1999; Liefertinck, 1966; own observations). As the Mediterranean Sea also inhibits geographic overlap between this pair of populations, their score is unlikely to surpass two points. While it may therefore be appropriate to treat these as subspecies *C. m. mercuriale* (Charpentier, 1840) and *C. m. hermeticum* (Selys, 1872) respectively, additional research would first be warranted.

Ecology and status

The species *C. castellani* has a scattered distribution, being more frequent along the coast and at lower el-

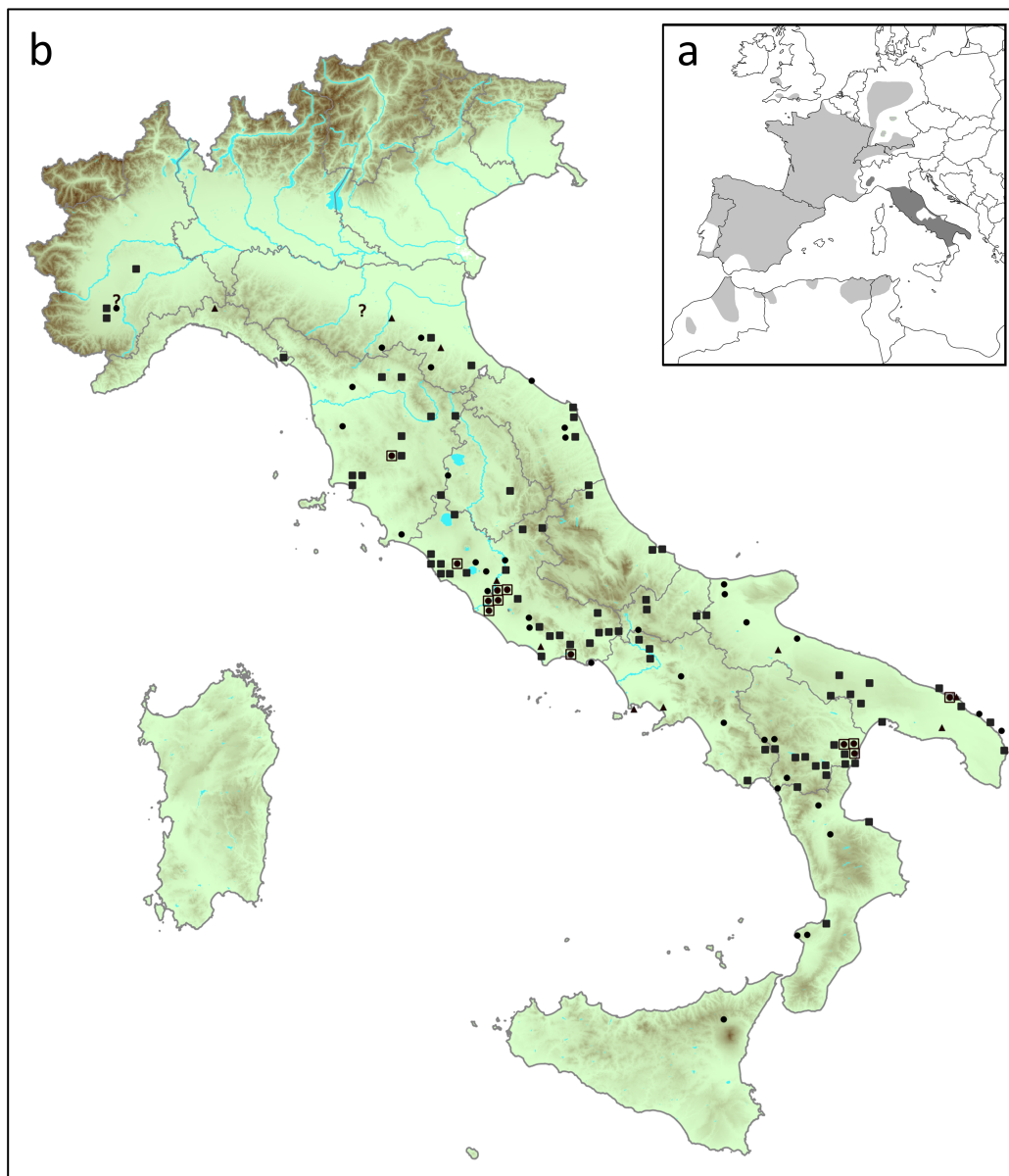


Figure 4. Distribution of *C. castellani* (a) in dark grey relative to the current world range of *C. mercuriale* (in pale grey), and (b) on a 10 × 10 km UTM grid: triangles indicate records before 1950, dots from 1950 to 1999, squares from 2000 until 2020, squares with-dots from 2000 or later as well as an earlier period, and question marks indicate doubtful records.

evaluations, with no records over 1050 m above sea level. Its apparent scarcity is probably due to its narrow ecological requirements and the incomplete knowledge of odonate distributions in Italy. Nonetheless, knowledge of the Italian fauna has increased vastly in recent decades (La Porta et al., 2023; Riservato et al., 2014b), as shown by the increase of grid squares occupied by the species in the last twenty years.

Ecology and phenology appear comparable to *C. mercuriale*. Habitats include runnels, small streams (often close to the source), and irrigation ditches, which are mostly slow-flowing and can have strongly fluctuating levels (Buchwald, 1994; Fabbri, 2018). These water bodies are generally unshaded, rich in aquatic and riparian vegetation, and often located in extensively managed farm- or grassland. As reported for *C. mercuriale* in North Africa (Mahdjoub et al., 2015), the species occasionally has two generations in a year, although adult numbers in autumn may be less than 10% of those at the same site in spring (Fabbri, 2018).

The status of *C. mercuriale* on the global IUCN Red List of Threatened Species, which still includes *C. castellani*, as well as on the Italian Red List, which now represents the global status of *C. castellani*, is Near-Threatened (Riservato et al., 2014a). No long-term data exist on population trend, although several reports of local extinction in intensively urbanised and cultivated areas due to habitat degradation, fragmentation, or loss have been reported (Fabbri, 2018; La Porta & Goretti, 2020). The decline of *C. mercuriale* in the last decade is inferred as 25% in France and nearly 78% in Iberia, while the North African populations are classified as Endangered (Ferreira et al., 2015). As the ecology of *C. castellani* is similar, as are ecological impacts in Italy (e.g. habitat loss due to agricultural practices, more frequent and harsher droughts due to climate warming), a recent decline between 30% and 50% is considered likely. This would qualify *C. castellani* for the threat status Vulnerable, as will be proposed during the upcoming review of the European Red List (Geert De Knijf, pers. comm.).

The European Union's Habitats Directive (Council Directive 92/43/EEC), designed to protect species and habitats of interest to the European community, includes *C. mercuriale* in Annex II. The habitat core areas of species included in Annex II of the directive must be designated as Special Areas of Conservation (SACs), included in the Natura 2000 network, and managed in accordance with the ecological needs of the species.

When species are split into multiple species (e.g. when subspecies are elevated to species rank), these generally inherit the conservation category of the original species (see DG Environment, 2017 for a long list of examples). There is no doubt that when the Directive was drafted and amended before, *C. castellani* was treated as a subspecies of *C. mercuriale* (see Askew, 1988; Carfi & Terzani, 1993; Castellani, 1954; Conci, 1949; Gianti, 2001; Dijkstra & Lewington, 2006) and must thus be retained on Annex II as a separate species too.

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Supplementary material

Supplementary table 1. Multi-locus molecular dataset of the *Coenagrion mercuriale* group. Haplotype and sampling details for each considered sequence are reported. The letters a and b indicate the unphased versions of the same sequenced marker.