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NOTA BREU

Re-instatement of the species name *Callirhytis erythrosoma* (Dettmer, 1933), with comments on other *Callirhytis* species (Hymenoptera: Cynipidae)

Restitució del nom de l’espècie *Callirhytis erythrosoma* (Dettmer, 1933), amb comentaris sobre altres espècies de *Callirhytis* (Hymenoptera: Cynipidae)

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Förster (1869) first described *Callirhytis* to include some European gall forming cynipid species that uniquely present transversely striated sculpturing on the scutum and malar sulcus. Since then some authors, without considering these diagnostic characters, have treated *Callirhytis* as a subgenus of *Andricus*: *Andricus* (*Callirhytis*) possessing simple tarsal claws, while the nominal subgenus *Andricus* (*Andricus*) having tarsal claws with a basal lobe (e.g. Mayr 1881; Ashmead 1885; Cameron 1893; Dalla Torre 1893; Kieffer 1897-1901). Later, Mayr (1902) restored the generic status of *Callirhytis* and Dalla Torre & Kieffer (1910) followed suit, as the tarsal claws are not a diagnostic character in *Callirhytis* (Nieves-Aldrey 1992). Weld (1922, 1926, 1952) included many Nearctic species in this genus, again ignoring the diagnostic characters given by Förster (1869), and as result of this *Callirhytis* has become a very chaotic genus (herein termed *Callirhytis* «sensu lato»). Melika & Abrahamson (2002) pointed out that of the 115 Nearctic species of *Callirhytis* named in Burks (1979), only 15 are *Callirhytis* «sensu stricto». Consistent with this, in recent years two new genera (*Zapatella* Pujade-Villar & Melika, 2012 and *Melikaiella* Pujade-Villar, 2014) have been described to include some of these incorrectly assigned Nearctic *Callirhytis* (Pujade-Villar *et al*. 2012, 2014), and further revisionary work on Nearctic *Callirhytis* is ongoing. According to Pénzes *et al.* (2018), a single *Callirhytis* species also occurs in the Eastern Palaearctic (*C. glanduliferae* Monzen, 1953).

Callirhytis is represented in the Western Palaearctic region by six species (Pujade-Villar *et al*., 2005): four are present in western and central Europe (*C. bella* (Dettmer, 1930); *C. erythrocephala* (Giraud, 1959); *C. glandium* (Giraud, 1959) and *C. rufescens* (Mayr, 1882)), and two species are known from the Caucasus region (*C. comantis* Belizin & Maisuradze, 1961 and *C. reticulatus* Belizin & Maisuradze, 1961). The sexual generations of the European species induce summer galls in twigs (rarely in buds) of oaks belonging to *Quercus* section *Quercus* and the asexual generations induce galls inside acorns of *Quercus* section *Cerris* oaks; larvae can diapause in these asexual galls for between one to eight years (Barbotin† *pers. comm*., after Nieves-Aldrey 1992).

Both generations are known for *C. glandium* and *C. rufescens* following Barbotin’s experimental studies, published by Nieves-Aldrey (1992). *Callirhytis comantis* is known only from its sexual generation and *C. reticulatus* from its asexual generation, and this pairing could represent alternate generations of the same biological species (Maisuradze 1961). The taxonomic status of *C. bella* is uncertain. This species, known only from females of the suspected sexual generation, emerges in April-May from cryptic galls located inside small buds on *Q. robur* and *Q. petraea*. It has rarely been collected, only being known from the Netherlands and British Islands (Nieves-Aldrey 1992) and France (new record; galls and adults collected from «Reserve Naturelle Forêt de la Massane/Banyuls Sur Mer»). Morphologically it is very similar to the sexual generation of *C. glandium*, from which it can only be distinguished through having smaller and more separated antennae as well as by slight differences in pronotal and mesopleural sculpturing (Nieves-Aldrey 1992). However, the sexual generation of *C. glandium* emerges much later in late July and August. This early emergence of *C. bella* is more typical of asexual forms of European *Callirhytis*, although its host plant association and morphology imply it is a sexual generation. Finally, *C. hartigi* Förster, 1869 was proposed as the possible sexual generation of *C. erythrocephala* by Nieves-Aldrey (1992) and was formally synonymized with *C. erythrocephala* by Pujade-Villar *et al.* (2005) after unpublished studies by both Folliot (in 1964-1967) and Barbotin† (in 1971-1986) independently closed this species’ life-cycle.

However, the asexual generation of *C. erythrocephala* as defined by Nieves-Aldrey (1992) presents some taxonomic issues. Dettmer (1933) described two asexual species: *Callirhytis erythrosoma* and *C. erythrostoma*. Both were synonymized as being the asexual generation of *C. erythrocephala* (Giraud, 1859) by Nieves-Aldrey (1992), although this was contrary to Barbotin’s unpublished experimental notes stating that both species induce different asexual galls. *Cal-*

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lirhytis erythrocephala (= *C. erythrostoma*) produces a coalescent and hard almond-shaped gall within the endocarpium of an acorn resulting from the fusing of multiple unilocular larval cells into one large agglomerate, while in contrast *C. erythrosoma* induces a hemispherical group of gall chambers which can usually be separated into individual larval chambers (Pujade-Villar *et al*., 2005). The asexual females of both forms are similar but according Barbotin's† notes can be easily distinguished: females reared from galls of *C. erythrosoma* are red-yellowish while those reared from the galls of *C. erythrocephala* have a black mesosoma. One way to resolve the status of these species requires experimental rearings that follow individuals through multiple generational cycles, which is a long and difficult process. Alternatively, a simpler method examining DNA sequence variation can be employed; recent work has demonstrated the efficacy of this method for discriminating among sister species in cynipids and matching otherwise unpaired generations (e.g. Stone *et al*. 2008; Nicholls *et al*. 2012, 2018).

To this end, DNA was extracted from two adult individuals of each asexual European *Callirhytis* species previously recognised in the literature as being morphologically distinct: *C. erythrostoma*, *C. erythrosoma*, *C. glandium* and *C. rufescens*. The adults of *C. erythrostoma* and *C. erythrosoma* were reared from *Q. cerris* acorns collected at the same locality, St Malo in north-western France, so provide a test of the genetic distinctiveness of these two morphotypes in sympatry. In addition, five larvae dissected from *Q. cerris* acorns in Hungary (two individuals) and Denmark (three individuals) were also extracted. A 433 base pair section of the mitochondrial cytochrome *b* gene was sequenced for each individual, as this gene has been demonstrated to provide species-level resolution within cynipids (Nicholls *et al*. 2012; Tang *et al.* 2016a, b; Cerasa *et al.* 2018; Nicholls *et al.* 2018; Tang *et al*. 2020). Variation within and between putative species was calculated, and a neighbour-joining tree was constructed using HKY genetic distances and rooted using the Nearctic species *Melikaiella tumifica* (GenBank accession MG821060).

The genetic results support the distinctiveness of the two species *C. erythrocephala* (= *C. erythrostoma*) and *C. erythrosoma*, as suggested by Pujade-Villar *et al.* (2005), although they are each other's closest relative (Figure 1). Variation between sympatric individuals of the two species averaged 2.4% (see Table 1), a value comparable to between-species variation both in other cynipid species (Nicholls *et al*. 2012; Cerasa *et al*. 2018; Nicholls *et al*. 2018) and among insects in general (Hebert *et al*., 2003). All the sequenced larvae appeared to be from the species *C. erythrosoma*, and within this species the divergence associated with geographically-separated samples was low (on average 0.5%; range 0-0.9%), again consistent with there being two genetically discrete species previously included in the *C. erythrocephala* complex. All new sequences are deposited in GenBank, accessions MT152275-MT152287.

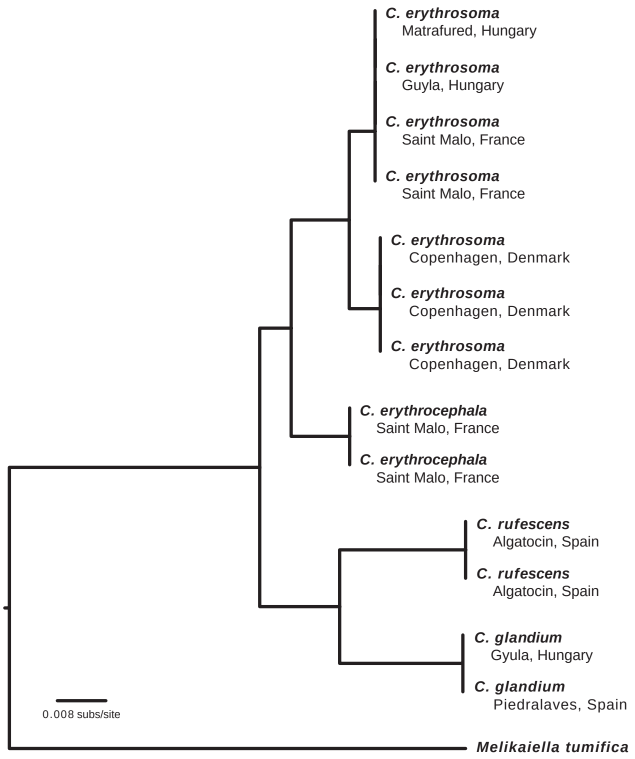

Figure 1. Neighbour-joining tree based upon cytochrome *b* gene sequences obtained from the four *Callirhytis* species present in Europe and with known asexual generations. Terminals are labelled with their species name and collection location.

In view of these results we formally re-instate the name *C. erythrosoma* to species rank. Additionally, following the discussion presented by Pujade-Villar *et al*. (2005), the sexual form of *C. erythrocephala* corresponds to the species originally described as *C. hartigi* whereas the sexual form of *C. erythrosoma* is the morphologically distinct but as yet undescribed generation illustrated in Figure 4 of Pujade-Villar *et al*. (2005). We note as well that these two sexual forms differ

Table 1. Pairwise genetic distances among the four *Callirhytis* species present in Europe and known from their asexual generations, based upon 433 base pairs of the cytochrome *b* gene using a HKY correction. Values on the diagonal indicate average percentage variation among individuals of the same species; off-diagonal values indicate between-species distances

	C. erythrosoma	*C. erythrocephala*	*C. rufescens*	*C. glandium*
C. erythrosoma	0.5	—	—	—
C. erythrocephala	2.4	0.0	—	—
C. rufescens	5.3	5.1	0.0	—
C. glandium	5.3	4.6	4.1	0.0

to a greater extent than the recognised species pair of *C. bella* and *C. glandium*.

To identify the European species (both sexual and asexual forms) we establish the following key:

1 Asexual females; robust specimens (body length 2.5-3.0 mm). Gena strongly broadened behind eye; head transverse, dull rugose; scutum with strong transverse irregular striae; galls exclusively in acorns on oaks belonging to *Quercus* section *Cerris* (*Q. cerris*, *Q. suber*, *Q. ilex*, *Q. macrolepis* and occasionally *Q. coccifera*) 2

– Sexual females and males; non-robust specimens (body at most 2.5 mm). Gena slightly broadened behind eye; head rounded or taller than broad in front view, coriaceous, with less dull sculpture; scutum with more delicate transverse striae; galls in twigs or buds on oaks of *Quercus* section *Quercus* (*Q. faginea*, *Q. pubescens*, *Q. pyrenaica*, *Q. robur*, among others)...5

2 Tarsal claws with basal lobe. Radial cell 3.0 times as long as broad; 2nd abscissa of radius slightly curved. Scutum with strong transverse regular striae. Body almost completely black or dark reddish brown............. *C. glandium*

– Tarsal claws simple or with very weak basal lobe. Radial cell longer, 3.5 times as long as broad; 2nd abscissa of radius nearly straight. Scutum with less strong transverse striae, usually irregular, waved and/or with anastomoses in posterior 1/2-1/3. Body colour variable, from almost entirely reddish yellow to predominantly black.............3

3 Body yellow-red to amber. Frons and vertex microreticulate, without strong branched striae. Notauli complete, sometimes less distinct in anterior 1/3. Scutum with less marked regular transverse striae. Tarsal claws simple. Galls on *Q. suber* and *Q. ilex* .... *C. rufescens*

– Body predominantly black or completely yellow-red. Frons and vertex dull rugose with strong striae. Notauli incomplete, invisible in anterior 1/3. Scutum with strong transverse waved, irregular rugae, with anastomoses posteriorly. Tarsal claws without or with very weak basal lobe. Galls on *Q. cerris* 4

4 Mesosoma completely black. OOL subequal to LOL. Vertex with strong rugae, inter-ocellar area always with linear elements .... *C. erythrocephala*

– Mesosoma red-yellowish. OOL longer than LOL. Vertex coriaceous sometimes with weak rugae, inter-ocellar area occasionally with linear elements .... *C. erythrosoma*

5 Tarsal claws toothed, with an acute basal lobe.................
.... *C. bella* and *C. glandium*
[*C. bella* emerge from inconspicuous bud galls and *C. glandium* from twigs]

– Tarsal claws simple or with blunt basal lobe..................6

6 Body with amber colour. Notauli complete, sometimes weakly impressed on the anterior part of the mesoscutum. Radial cell relatively short (3.1-3.3 times longer than wide). Males with large ocelli (OOL equal to 0.5 times the diameter of a ocellus) and transfacial line much shorter than the height of a compound eye............... *C. rufescens*

– Predominantly reddish-brown colour. Notauli incomplete, invisible on the anterior third of the mesoscutum. Radial cell longer (about 3.7 times longer than wide). Males with relatively smaller ocelli (OOL 0.9-1.2 times as long as the diameter of the lateral eye) and transfacial line almost equal to the height of a compound eye...........................7

7 Head around 2.0 times as broad as long in dorsal view. Scutellar foveae separated by a wide central area. Females POL 1.5-1.6 times as long as OOL and F1 3.0 times as long as broad. Males with notauli reaching 2/3 the length of the mesoscutum and OOL shorter than diameter of lateral ocellus .... *C. erythrocephala*

– Head broader, less transverse. Scutellar foveae separated by a point or a little carina. Females POL subequal to OOL and F1 around 2.1 times as long as broad. Males with notauli reaching 1/3 the length of the mesoscutum and OOL slightly longer than diameter of lateral ocellus ....
.... *C. erythrosoma*

Finally, we provide comment on the status of one further *Callirhytis* species, *C. cameroni*, a species known from leaf galls on *Q. salicifolia* (*Quercus* section *Lobatae*) in Panama (Medianero & Nieves-Aldrey 2014). As with most other Nearctic *Callirhytis*, it lacks the diagnostic characters of *Callirhytis* «sensu stricto»; it is currently classified as *Callirhytis* «sensu lato» only based on the apparently incorrect characters of Weld (1952). According to the original description, *C. cameroni* has reticulate sculpture on the third and following metasomal tergites, and such sculpturing never occurs in *Callirhytis* «sensu stricto». Reticulate or strongly alveolate metasomal sculpturing is rare in Nearctic Cynipini and only occurs in the genus *Melikaiella* Pujade-Villar, 2014. *Callirhytis cameroni* also shares other characters typical of *Melikaiella* (see Pujade-Villar *et al.*, 2014): lower facial carinae present only along the malar sulcus (carinae radiating from clypeus present in the entire malar area and extending on the lower face up to toruli in *Callirhytis*), a very strong irregular rugose sculpture on mesoscutum (not transversely carinated as in *Callirhytis*) with coriaceous interspaces (interspaces smooth and shiny in *Callirhytis*), circumscutelar carina (absent in *Callirhytis*) and mesopleuron having coriaceus reticulate sculpture (with transverse and interrupted striae in *Callirhytis*). The host plant of *C. cameroni* occurs on *Quercus* section *Lobatae* section as is the case with other *Melikaiella* species (sections *Quercus* and *Cerris* are hosts of true *Callirhytis*). Accordingly, we transfer *C. cameroni* to the genus *Melikaiella*, with the new name *Melikaiella cameroni* (Medianero & Nieves-Aldrey, 2014) **n. comb.**

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