# Review of the fish parasitic genus Ceratothoa Dana, I852 (Crustacea, Isopoda, Cymothoidae) from South Africa, including the description of two new species 

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#### Abstract

The genus Ceratothoa Dana, 1852 is revised for South African waters and re-diagnosed. Ceratothoa retusa (Schioedte \& Meinert, 1883) is recorded from the eastern coast, and Ceratothoa africanae sp. n. and C. famosa sp. n. are described; C. imbricata (Fabricius, 1775) and C. trigonocephala (Leach, 1818), are redescribed, revised and excluded from the South African fauna. Ceratothoa africanae sp. n. can be distinguished by the stout body shape of the female; triangular cephalon with a pointed rostrum; short uropods which do not extend past the pleotelson; large carinae on the pereopod basis; a broad pleon; and large medial lobes on female pleopods. Ceratothoa famosa $\mathbf{s p}$. $\mathbf{n}$. is characterised by the long rectangular body shape; pereonite 1 with a raised medial protrusion; narrow antenna with antennule article 1 expanded; uropods which reach the posterior margin of the pleotelson; narrow rami on uropods; and no appendix masculina on pleopod 2 of the male specimens.


## Keywords

Isopod, cymothoid, buccal-cavity, fish parasite, tongue-biter, Indian Ocean

## Introduction

Cymothoid isopods are obligate parasites of both freshwater and marine fishes, where they will attach to the external surfaces, gills or inside the buccal-cavity of their fish host (Kensley and Schotte 1989, Trilles 1991). These isopods are economically important parasites as they have been shown to cause detrimental effects on fish in captivity including growth inhibition, malnutrition, anaemia and death in smaller fish (Romestand 1979, Bragoni et al. 1984, Adlard and Lester 1994, Horton and Okamura 2001, Mladineo 2002, Ravi and Rajkumar 2007). One of the most common genera of tongue-biters (cymothoids found inside the buccal-cavity of the fish host, attached to the tongue) in southern Africa is Ceratothoa Dana, 1852.

Very little is known about the cymothoid isopods from southern Africa and the western Indian Ocean (Kensley 1978, 2001). Recently, two buccal attaching genera have been reviewed; Hadfield et al. (2010) revised the monotypic genus Cinusa Schioedte \& Meinert, 1884, endemic to this region and Hadfield et al. (2013) reviewed Cymothoa Fabricius, 1787 from the southwestern Indian Ocean.

Ceratothoa has long been considered to have three species in South Africa: Ceratothoa imbricata (Fabricius, 1775), C. retusa (Schioedte \& Meinert, 1883) and C. trigonocephala (Leach, 1818) (see Kensley 2001). In the present study, none of the material agreed with the descriptions of C. imbricata and C. trigonocephala, and no positive identification for these two species in South Africa could be made. These species are therefore excluded from the South African fauna. However, sampling revealed two new species from the region, leaving the total at three species of Ceratothoa in South Africa.

## Methods

Type material for Ceratothoa imbricata and C. trigonocephala were borrowed from the Natural History Museum, UK. All available material from Iziko South African Museum labelled as a Ceratothoa species was borrowed with additional specimens being obtained from fish hosts held in the South African Institute for Aquatic Biodiversity (SAIAB), Grahamstown, South Africa.

New material was collected along the south coast of South Africa by the FRS Africana and from intertidal rock pools at Tsitsikamma National Park.

Isopods were processed according to the techniques described in Hadfield et al. (2010, 2011, 2013). Species descriptions were prepared in DELTA (Descriptive Language for Taxonomy, see Coleman et al. 2010) using a general Cymothoidae character set. Classification follows Brandt and Poore (2003).

Host nomenclature and distribution are from FishBase (Froese and Pauly 2013).

Synonymies: Those records that we have been unable to confirm directly from specimens or the published figures, or that we otherwise have reasonable doubts about, have been removed from the synonymy.

Abbreviations. BMNH—British Museum, Natural History, UK (now NHMUK); MNHN—Muséum National d'Histoire Naturelle, Paris; NHMUK—Natural History Museum, UK; SAIAB—South African Institute for Aquatic Biodiversity, Grahamstown; SAM—South African Museum, Cape Town; SMNH—Swedish Museum of Natural History, Stockholm; ZMUC—Zoological Museum, University of Copenhagen; TL—total length; W—width.

## Taxonomy

## Family Cymothoidae Leach, 1814

Genus Ceratothoa Dana, 1852
http://species-id.net/wiki/Ceratothoa
Ceratothoa Dana, 1852: 303; Miers 1876: 104-105; Haswell 1882: 282; Schioedte and Meinert 1883: 322-323; Richardson 1905: 233-234; Bowman 1978: 217218; Brusca 1981: 177-178; Bruce and Bowman 1989: 1-2; Horton 2000: 1041; Martin et al. 2013: 396.
Codonophilus Haswell, 1881: 471.- 1882: 283; Hale 1926: 201, 223.
Rhexana Schioedte \& Meinert, 1883: 289-290.
Cteatessa Schioedte \& Meinert, 1883: 296-297.
Meinertia Stebbing, 1893: 354.- 1900: 642; 1910a: 103; Richardson 1905: 236-237; Menzies 1962: 116; Schultz 1969: 156.
Rhexanella Stebbing, 1911: 179.
Not Ceratothoa.- Dana 1853: 747; Richardson 1905: 236; Schultz 1969: 155; Kussakin 1979: 287 [= Glossobius Schioedte \& Meinert, 1883].

Type species. Dana (1852) included two species, Cymothoa gaudichaudii Milne Edwards, 1840 and Cymothoa parallela Otto, 1828 in his new genus without designating a type species (Bowman 1978). Bowman (1978) resolved the generic name, concluding that Ceratothoa had priority over other names that had been in use, but did not designate a type species. The whereabouts of the C. parallela type specimen is unknown, and is thought to no longer be extant (Bruce and Bowman 1989, Horton 2000, Martin et al. 2013). Horton (2000), however, designated a neotype of C. parallela from Oran (but without a redescription) after Schioedte and Meinert (1883) referred to it as a "specim. typ.". The syntype female for C. gaudichaudii is in pieces (the male syntype is still intact) and held at the Muséum National d'Histoire Naturelle, Paris (Trilles 1973, Hadfield pers. obs.). A type species should ideally be designated only when one or both of the species is fully redescribed and its identity and type material clearly established.

Diagnosis. Body narrow, strongly vaulted, 2.1-2.9 times as long as wide, widest at pereonite 5 . Cephalon triangular, with blunt rostrum, anterior margin ventrally directed, posterior margin straight. Antennular bases in contact, broad and expanded, subequal to antenna. Eyes distinct. Mandible not expanded; mandible palp article 2 longer than article 3. Maxilla medial lobe partly fused, prominent nodulose spines on each lobe. Maxillule with 4 terminal spines. Maxilliped article 3 with 2 recurved spines, with oostegite lobe. Pereonite 1 anterolateral angles extensions encompassing cephalon. Pereonites 6 and 7 posterolateral margins not produced. Pereonite 7 extends past pleonite 1. Pleon subequal or narrower than pereon. Pleonite 1 width narrower than other pleonites, pleonites $2-5$ subequal in width. Pleotelson narrower than pleonites. Coxae 5-7 visible, reniform, often produced and rounded, shorter than somite. Brood pouch from coxae 1-4 and 6, posterior pocket absent. Pereopods 5-7 basis with large blade-like carina, without robust setae. Pereopod 7 slightly larger or more than 1.5 times longer than pereopod 1. Pleopods from dorsal view not visible, decreasing in size posteriorly. Pleopods $1-5$ with small pleats or pockets, with proximomedial lamellar lobe (more pronounced in pleopods 3-5), peduncle lobes on the lateral margin absent. Uropod rami short, not extending past posterior margin of pleotelson, subequal.

Remarks. Ceratothoa can best be identified by the triangular cephalon, contiguous antennular bases, pleonite 1 narrower than the other pleonites, elongate body (2.1-2.9 times as long as wide), and subequal uropod rami which extend to the posterior margin of the pleotelson. Bruce and Bowman (1989) highlighted that Ceratothoa has unique pereopod morphology, with most species having prominent expansions on the basis of the posterior pereopods (pronounced carina), except Ceratothoa gilberti (Richardson, 1904) that has no expansions on any of the pereopods. Furthermore, the ischium of the posterior pereopods is also expanded in some species such as Ceratothoa guttata (Richardson, 1910) (see Bruce and Bowman 1989) and C. carinata (Bianconi, 1869) (see Martin et al. 2013).

The most recent reviews of this genus are those of Bruce and Bowman (1989) and Martin et al. (2013). Meinertia Stebbing, 1893 and Codonophilus Haswell, 1881 were placed into synonymy with Ceratothoa, the senior available name by Bowman (1978), and Bruce and Bowman (1989) synonymised Cteatessa Schioedte \& Meinert, 1883 and Rhexanella Stebbing, 1911 with Ceratothoa. Glossobius is distinct from Ceratothoa and is considered a valid genus which includes species associated with pelagic beloniform fishes (Exocoetidae, Hemirhamphidae).

Relationships. Phylogenetic relationships of the cymothoid genera remain unassessed, other than comments given by Brusca (1981), Bruce and Bowman (1989), and the molecular analyses (using small data sets) of Ketmaier et al. (2008) and Jones et al. (2008). Brusca (1981) postulated that there were three evolutionary "lineages" within Cymothoidae based on their attachment sites on the hosts (external surfaces, buccal+gill, and the freshwater flesh burrowing genera). Both Ketmaier et al. (2008) and Jones et al. (2008) later demonstrated that these lineages could not necessarily be upheld.

In a preliminary phylogenetic analysis using 23 cymothoid genera, with Rocinela Leach, 1818 (Aegidae) as the outgroup (Hadfield 2012), the buccal-cavity isopods grouped together in a clade based on these genera having a cephalon encompassed by the anterolateral margins of pereonite 1 , pereopods 5-7 with a large blade-like carina on the basis, and a partly fused maxilla medial lobe (with the exception of Glossobius which has lobed anterolateral margins and a distinct maxilla mesial lobe and Lobotho$\operatorname{rax}$ Bleeker, 1857 which has no carina on the basis).

Ceratothoa is most closely related to Glossobius and this was shown in the preliminary study where the two genera grouped as sister taxa (Hadfield 2012). These genera share many similar characteristics such as the antennular bases being in contact (the apomorphic character for this clade); expanded antennules; antennules subequal to antennae; maxilliped article 3 with 2 recurved spines; and no peduncle lobes on the pleopods. Ceratothoa is distinguished from Glossobius by having distinct eyes; maxilla medial lobe partly fused rather than distinct; maxilliped with only one oostegite lobe compared to the two in Glossobius; anterolateral margins of pereonite 1 extended (not lobed as in Glossobius); and uropod rami are subequal.

## Ceratothoa retusa (Schioedte \& Meinert, 1883)

http://species-id.net/wiki/Ceratothoa_retusa
Ceratothoa retusa Schioedte \& Meinert in Hilgendorf 1879: 847 [nomen nudum].
Cteatessa retusa.- Schioedte \& Meinert, 1883: 297-299, tab. XI (Cym. XVIII) Figs 11-13;
Stebbing 1908: 424; Barnard 1925: 393.- 1940: 491; Nierstrasz 1931: 131; Trilles 1986: 625, tab. 1; 1994: 130; 2008: 23; Kensley 1978: 79-80, Figs 32 (g-h).
Codonophilus hemiramphi Pillai, 1954: 14-15 [nomen dubium].
Ceratothoa hemiramphi.- Trilles 1994: 120; Kensley 2001: 232.
Ceratothoa retusa.- Bruce and Bowman 1989: 8-12, Figs 5-8; Kensley 2001: 232; Trilles et al. 2011: 446-459; Hadfield et al. in press.

Distribution. Indian Ocean—records from Mozambique, South Africa, Red Sea, India, Indonesia and northern Australia (see Hadfield et al. in press).

Hosts. Hemirhamphidae buccal-cavity—Hemirhamphus far (Forsskål, 1775) and H. robustus Günther, 1866 (see Hadfield et al. in press).

Remarks. Ceratothoa retusa can be identified by the large pereonite 1 with an anterolateral ridge and small cephalon sunken into pereonite 1 . Pereonite 1 is deeply concave with anterolateral margins which almost extend to the tip of the cephalon. The pleotelson is broader than long and the uropods extend past the pleotelson margin (Hadfield et al. in press). This species was redescribed in detail by Hadfield et al. (in press) and has been shown to have a variable morphology depending on the sampling location of the specimen.

## Ceratothoa africanae sp. n.

http://zoobank.org/B4BA5F68-2070-4464-88FB-B27356761920
http://species-id.net/wiki/Ceratothoa_africanae
Figs 1-7, 21

Material examined. Holotype. Female ( $29 \mathrm{~mm} \mathrm{TL} ; 15 \mathrm{~mm}$ W), collected from a trawl ( $34^{\circ} 26^{\prime} \mathrm{S}, 24^{\circ} 13^{\prime} \mathrm{E}$ ) along the south coast of South Africa from the buccal-cavity of Spondyliosoma emarginatum, 30-04-2003, coll. N.J. Smit (SAM A45937; HP 221).

Paratypes. All from the buccal-cavity of Spondyliosoma emarginatum and collected while trawling ( $34^{\circ} 26^{\prime}$ S, $24^{\circ} 13^{\prime} \mathrm{E}$ ) along the south coast of South Africa (30-04-2003), coll. N.J. Smit: Three females ( $22-26 \mathrm{~mm} \mathrm{TL} ; 12-15 \mathrm{~mm}$ W), three males ( $11-13$ $\mathrm{mm} \mathrm{TL} ; 5-6 \mathrm{~mm}$ W), one dissected female ( 30 mm TL; 15 mm W), one dissected male ( 16 mm TL; 7 mm W) (SAM A45938; HP 221).

Other material. In the possession of authors at NWU. From the the buccal-cavity of Spondyliosoma emarginatum: Algoa Bay ( $33^{\circ} 51^{\prime} \mathrm{S}, 25^{\circ} 52^{\prime} \mathrm{E}$ ), 1978: female ( 28 mm TL; 12 mm W). Eastern Cape, no date: female ( $20 \mathrm{~mm} \mathrm{TL} ; 11 \mathrm{~mm}$ W), male ( 7 mm TL; 3 mm W). Tsitsikamma Grootbank, Sout River ( $34^{\circ} 1^{\prime}$ S, $23^{\circ} 28^{\prime} \mathrm{E}$ ), September 1993: female ( $19 \mathrm{~mm} \mathrm{TL} ; 8 \mathrm{~mm} \mathrm{~W}$ ).

Ovigerous female holotype. Length 19-29 (23.4) mm, width 8-15 (12.5) mm.
Body ovoid, 1.5 times as long as greatest width, dorsal surfaces smooth and polished in appearance, widest at pereonite 4 , most narrow at pereonite 1 , lateral margins posteriorly ovate. Cephalon 0.6 times longer than wide, visible from dorsal view, triangular. Frontal margin rounded to form blunt rostrum. Eyes oval with distinct margins. Pereonite 1 with slight indentations, anterior border straight, anterolateral angle with small distinct anterior projection which does not extend past the eyes, posterior margins of pereonites smooth and straight. Coxae $2-3$ with posteroventral angles not visible; 4-7 rounded. Pereonites 1-4 increasing in length and width; 5-7 decreasing in length and width; becoming more progressively rounded posteriorly. Pleon with pleonite 1 same width as other pleonites, visible in dorsal view; pleonites posterior margin smooth, mostly concave; posterolateral angles of pleonite 2 narrowly rounded, not posteriorly produced. Pleonites 3-5 similar in form to pleonite 2 . Pleonite 5 with posterolateral angles free, not overlapped by lateral margins of pleonite 4 , posterior margin with 2 indented points. Pleotelson 0.5 times as long as anterior width, dorsal surface smooth, lateral margins posteriorly narrow, posterior margin evenly rounded, without median point.

Antennule more stout than antenna, comprised of 7 articles; peduncle articles 1 and 2 distinct and articulated; article 20.9 times as long as article 1 ; article 30.3 times as long as combined lengths of articles 1 and 2, 0.7 times as long as wide; flagellum with 4 articles, extending to anterior of pereonite 1 . Antenna comprised of 8 articles. Antenna peduncle article 31.5 times as long as article 2, as long as wide; article 40.8 times as long as wide, 0.8 times as long as article 3; article 50.5 times as long as article 4, 0.7 times as long as wide. Antenna flagellum with 3 articles, last article terminating in no setae, extending to anterior margin of pereonite 1 . Anterior margin acute, with small median point. Mandibular process ending in an acute incisor, with no simple setae,
mandible palp article 2 and 3 without setae. Maxillule simple with 4 terminal robust setae. Maxilla mesial lobe partly fused to lateral lobe; lateral lobe without simple setae, 3 recurved robust setae; mesial lobe with 6 large recurved robust setae and no simple setae. Maxilliped covered in pectinate scales and comprised of 3 articles, with lamellar oostegite lobe, palp article 2 without simple setae, article 3 with 5 recurved robust setae. Oostegites margin covered in numerous plumose setae, attached to pereopods 2-5.

Pereopod 1 basis 1.6 times as long as greatest width; ischium 0.7 times as long as basis; merus proximal margin with bulbous protrusion; carpus with straight proximal margin; propodus 1.3 times as long as wide; dactylus slender, 1.1 as long as propodus, 2.4 times as long as basal width. Pereopod 2 propodus 1.4 as long as wide; dactylus 1.1 as long as propodus. Pereopods gradually increasing in size towards posterior and all without robust or simple setae. Pereopod 6 basis 1.3 times as long as greatest width, ischium 0.9 times as long as basis, propodus 1.4 as long as wide, dactylus 1.3 as long as propodus. Pereopod 7 basis 1.3 times as long as greatest width; ischium 0.7 as long as basis, without protrusions; merus proximal margin with slight bulbous protrusion, merus 0.3 as long as ischium, 0.4 times as long as wide; carpus 1.3 as long as ischium, without bulbous protrusion, 0.8 times as long as wide; propodus 0.7 as long as ischium, 1.4 times as long as wide; dactylus slender, 1.3 as long as propodus, 3 times as long as basal width.

Pleopods without setae, exopod larger than endopod. Pleopod 1 exopod as long as wide, lateral margin strongly convex, distally truncate, mesial margin weakly convex; endopod 1.2 times as long as wide, lateral margin convex, distally subtruncate, mesial margin straight; peduncle 3.3 times as wide as long, without retinaculae. Pleopods 2-5 similar to pleopod 1 and mesial margins becoming more strongly produced. Pleopods $3-5$ endopods proximal borders extending below exopod to peduncle. Large medial lobes present and increasing in size from pleopod 1 to 5 .

Uropod more than half the length of pleotelson, peduncle 0.7 times longer than rami, peduncle lateral margin without setae; rami not extending beyond pleotelson, marginal setae absent, apices narrowly rounded. Endopod apically slightly pointed, 3.8 times as long as greatest width, lateral margin straight, terminating without setae, mesial margin straight. Exopod not extending to end of endopod, 4.1 times as long as greatest width, apically rounded, lateral margin weakly convex, terminating with no setae, mesial margin straight.

Male. Length 7-14 (10.6) mm, width 3-7 (4.9) mm.
Males similar to females but much smaller. Body oval, 1.4 times as long as wide. Penis small, low tubercles. Pleopod 2 appendix masculina with parallel margins, 1.2 times as long as endopod, distally bluntly rounded.

Etymology. Named for FRS Africana, from which the species was collected, also acknowledging that this is the first Ceratothoa species to be described from Africa.

Distribution. Eastern Cape Province, South Africa: from Tsitsikamma to Algoa Bay.
Hosts. Found in the buccal-cavity, on the tongue of Spondyliosoma emarginatum (Valenciennes, 1830).

Prevalence. 9/17 (53\%) of Spondyliosoma emarginatum infected from the FRS Africana trawls, 4/68 (5.9\%) from the SAIAB collections.


Figure I. Ceratothoa africanae sp. n. female holotype ( 29 mm ) (SAM A45937): A dorsal view B anterodorsal view of pereonite 1 and cephalon $\mathbf{C}$ ventral view of cephalon $\mathbf{D}$ dorsal view of pleotelson $\mathbf{E}$ lateral view.


Figure 2. Ceratothoa africanae sp. n. female paratype ( 30 mm ) (SAM A45938): A antennule B antenna $\mathbf{C}$ mandible $\mathbf{D}$ tip of maxillule $\mathbf{E}$ maxillule $\mathbf{F}$ tip of maxilliped article $3 \mathbf{G}$ maxilla $\mathbf{H}$ tip of maxilla $\mathbf{I}$ maxilliped with oostegite $\mathbf{J}$ oostegites $\mathbf{K}$ uropod.


Figure 3. Ceratothoa africanae sp. n. female holotype ( 29 mm ) (SAM A45937): A pereopod 1 B pereo$\operatorname{pod} 2 \mathbf{C}$ pereopod $6 \mathbf{D}$ pereopod 7 .


Figure 4. Ceratothoa africanae sp. n. female paratype ( 30 mm ) (SAM A45938): A dorsal pleopod 1 B dorsal pleopod $2 \mathbf{C}$ dorsal pleopod $3 \mathbf{D}$ dorsal pleopod $4 \mathbf{E}$ dorsal pleopod $5 \mathbf{F}$ ventral pleopod 1 $\mathbf{G}$ ventral pleopod $2 \mathbf{H}$ ventral pleopod $3 \mathbf{I}$ ventral pleopod $4 \mathbf{J}$ ventral pleopod 5 .


Figure 5. Ceratothoa africanae sp. n. male paratype ( 14 mm ) (SAM A45938): A dorsal view B antero-dorsal view of pereonite 1 and cephalon $\mathbf{C}$ ventral view of cephalon $\mathbf{D}$ dorsal view of pleotelson $\mathbf{E}$ lateral view.


Figure 6. Ceratothoa africanae sp. n. male paratype ( 14 mm ) (SAM A45938): A antennule $\mathbf{B}$ antenna $\mathbf{C}$ maxillule $\mathbf{D}$ mandible $\mathbf{E}$ maxilla $\mathbf{F}$ tip of maxilliped $\mathbf{G}$ maxilliped $\mathbf{H}$ uropod $\mathbf{I}$ pereopod $1 \mathbf{J}$ pereopod 7 .


Figure 7. Ceratothoa africanae sp. n. male paratype ( 14 mm ) (SAM A45938): A dorsal pleopod 1 B dorsal pleopod $2 \mathbf{C}$ dorsal pleopod $3 \mathbf{D}$ dorsal pleopod $4 \mathbf{E}$ dorsal pleopod $5 \mathbf{F}$ ventral pleopod $1 \mathbf{G}$ ventral pleopod $2 \mathbf{H}$ ventral pleopod $3 \mathbf{I}$ ventral pleopod $4 \mathbf{J}$ ventral pleopod 5 .

Remarks. Ceratothoa africanae sp. n. can be distinguished by the stout body shape of the female; a pointed rostrum; short and stout antennae; uropods which do not extend past the pleotelson; a broad pleon; large medial lobes on female pleopods; and an appendix masculina on the second pleopod in male specimens. This species was compared to the known species from South Africa at the time (C. imbricata, C. retusa and C. trigonocephala) and found to be distinct. Upon comparisons to other known species worldwide, it was concluded to be a new species.

Ceratothoa africanae sp. n. differs from C. retusa in having a larger cephalon not sunken into pereonite 1 as seen in C. retusa; lacks the anterolateral ridge on pereonite 1 ; has shorter uropods which do not extend past the posterior margin of the pleotelson; and lacks the large extended anterolateral margins on pereonite 1 which extend more than half the length of the cephalon in $C$. retusa but less than half in $C$. africanae.

Ceratothoa africanae sp. n. shares many similarities with C. imbricata and C. famosa sp. n. Ceratothoa africanae resembles $C$. imbricata in having pereonite 1 longer than pereonites $2-4$ and both have two concave mediolateral indents on the pleonite 5 posterior margin, but differs in having a broader body, anterolateral angles on pereonite 1 which do not extend past the eyes as is seen in C. imbricata, shorter uropods that do not extend past the pleotelson and an acute cephalon anterior margin. The pleopods of $C$. africanae have a few smaller lobes and folds and the pereopod 6 and 7 merus is produced on both the anterior and posterior sides. Furthermore, C. africanae pereonite 7 does not overlap any pleonites and pereopods 1 to 3 have a smaller merus, but pereopods 4 to 7 are larger. Ceratothoa africanae and C. famosa (see below) differ in the number of setae on the mandibular palp (five on C. africanae and three on C. famosa); no setae on the maxilliped palp in C. africanae sp. n. but seven setae on C. famosa; and nine setae on C. africanae maxilla but ten on C. famosa. More differences are noted in the remarks on C. famosa sp. n.

## Ceratothoa famosa sp. n.

http://zoobank.org/6F47F60D-9157-446F-9A2E-8A189549F087
http://species-id.net/wiki/Ceratothoa_famosa
Figs 8-14, 21
? Meinertia imbricata.- Trilles 1972: 1248-1250, pl. II (10-11).

Material examined. Holotype. Female ( 23 mm TL; 10 mm W), collected from Tsitsikamma National Park ( $34^{\circ} 1^{\prime}$ 'S, $23^{\circ} 52^{\prime} \mathrm{E}$ ) along the south coast of South Africa from the buccal-cavity of Diplodus sargus capensis, March 2005, coll. K.A. Hadfield (SAM A45939).

Paratypes. All from Tsitsikamma National Park ( $34^{\circ} 1^{\prime}$ 'S, $23^{\circ} 52^{\prime}$ E), Western Cape Province. From the buccal-cavity of Diplodus sargus capensis: dissected female ( 27 mm TL; 12 mm W), dissected male ( 13 mm TL; 6 mm W), April 2009, coll. K.A. Hadfield (SAM A45940); female ( 17 mm TL; 7 mm W), males ( $7,14 \mathrm{~mm} \mathrm{TL} ; 3,6 \mathrm{~mm}$ W), March 2005, coll. K.A. Hadfield (SAM A45941).

From the buccal-cavity of Sparadon durbanensis: female ( $15 \mathrm{~mm} \mathrm{TL} ; 6 \mathrm{~W}$ ), male ( 5.5 mm TL; 2 mm W), April 2009, coll. K.A. Hadfield (SAM A45942).

Other material. In the possession of authors at NWU. From Diplodus sargus capensis: Cape Agulhas ( $34^{\circ} 49^{\prime} \mathrm{S}, 20^{\circ} 0^{\prime} \mathrm{E}$ ): female ( $18 \mathrm{~mm} \mathrm{TL} ; 7 \mathrm{~mm} \mathrm{~W}$ ), male ( $9 \mathrm{~mm} \mathrm{TL} ; 3 \mathrm{~mm}$ W). Kenton-on-sea ( $33^{\circ} 42^{\prime} \mathrm{S}, 26^{\circ} 41^{\prime} \mathrm{E}$ ): female ( $14 \mathrm{~mm} \mathrm{TL} ; 5 \mathrm{~mm} \mathrm{~W}$ ), male ( 5 mm TL ; 2 mm W), May 1974. Morgan Bay ( $32^{\circ} 42^{\prime} \mathrm{S} ; 2^{\circ} 8^{\circ} 20^{\prime} \mathrm{E}$ ): two females ( $10,12 \mathrm{~mm}$ TL; 3, 4 mm W), April 2003. Swartkops River Estuary ( $33^{\circ} 52^{\prime} \mathrm{S} ; 25^{\circ} 38^{\prime} \mathrm{E}$ ): female ( 10 mm TL; 3 mm W), male ( 6 mm TL; 2 mm W), July 1980. Transkei, between Goss Bay and Lupatana: female ( 10 mm TL; 4 mm W), September 1975. Transkei, Grosvenor Point ( $31^{\circ} 22^{\prime} \mathrm{S}, 29^{\circ} 53^{\prime} \mathrm{E}$ ): female ( 15 mm TL; 6 mm W ), male ( $6 \mathrm{~mm} \mathrm{TL} ; 2 \mathrm{~mm} \mathrm{~W}$ ), September 1975. Tshani ( $31^{\circ} 56^{\prime} \mathrm{S}, 29^{\circ} 12^{\prime} \mathrm{E}$ ): female ( $10 \mathrm{~mm} \mathrm{TL;} 3 \mathrm{~mm}$ W), June 1996.

From Diplodus cervinus hottentotus: Kleinemonde ( $33^{\circ} 32^{\prime} \mathrm{S}, 27^{\circ} 03^{\prime} \mathrm{E}$ ): female ( 25 mm TL; 6 mm W), male ( 5 mm TL; 2 mm W), June 1975; female ( 15 mm TL; 6 mm W), male ( 6 mm TL; 2 mm W), March 1975; Keiskamma River Mouth ( $33^{\circ} 16^{\prime} \mathrm{S}$, $27^{\circ} 29^{\prime} \mathrm{E}$ ): female ( 18 mm TL; 8 mm W), male ( 9 mm TL; 4 mm W), February 1976; Knysna ( $34^{\circ} 5^{\prime} \mathrm{S}$, $23^{\circ} 3^{\prime} \mathrm{E}$ ): female ( 19 mm TL; 7 mm W), male ( $8 \mathrm{~mm} \mathrm{TL} ; 3 \mathrm{~mm} \mathrm{~W}$ ), 1945-1969. Tsitsikamma National Park ( $34^{\circ} 1^{\prime} \mathrm{S}, 23^{\circ} 52^{\prime} \mathrm{E}$ ): female ( 20 mm TL; 7 W ), male ( 14 mm TL; 5 mm W), 17 juveniles, March 2007.

From Sparadon durbanensis: Cape Padrone, Eastern Cape ( $33^{\circ} 46^{\prime} \mathrm{S}, 26^{\circ} 28^{\prime} \mathrm{E}$ ): four pullus ( 5 mm TL; 2 mm W), July 1975 . Kleinemonde ( $33^{\circ} 32^{\prime} \mathrm{S}, 27^{\circ} 03^{\prime} \mathrm{E}$ ): five pullus ( $4 \mathrm{~mm} \mathrm{TL} ; 2 \mathrm{~mm} \mathrm{~W}$ ), February 1977. Knysna ( $34^{\circ} 5^{\prime} \mathrm{S}, 23^{\circ} 3^{\prime} \mathrm{E}$ ): two pullus ( 5 mm TL; 2 mm W), 1945-1965. Tsitsikamma National Park ( $34^{\circ} 1^{\prime} \mathrm{S}, 23^{\circ} 52^{\prime} \mathrm{E}$ ): female ( 26 mm TL; 12 W ), male ( 12 mm TL; 5 mm W), 35 juveniles, July 2008.

Ovigerous female holotype. Length 10-28 (16.9) mm, width 3-12 (6.0) mm.
Body rectangular, 1.7 times as long as greatest width, dorsal surfaces smooth and polished in appearance, widest at pereonite 4 and pereonite 5, most narrow at pereonite 7 , lateral margins slightly convex. Cephalon 0.8 times longer than wide, slightly visible in dorsal view, triangular. Frontal margin rounded to form blunt rostrum. Eyes irregular in outline. Pereonite 1 with median projection, anterior border straight, anterolateral angle with distinct anterior projection, posterior margins of pereonites smooth and slightly curved laterally. Coxae 2-3 with posteroventral angles not visible; 4-7 rounded. Pereonites $1-5$ increasing in length and width; 6-7 decreasing in length and width; becoming more progressively rounded posteriorly. Pleon with pleonite 1 same width as other pleonites, visible in dorsal view; pleonites posterior margin smooth, mostly concave; posterolateral angles of pleonite 2 narrowly rounded, not posteriorly produced. Pleonites 3-5 similar in form to pleonite 2. Pleonite 5 with posterolateral angles free, not overlapped by lateral margins of pleonite 4, posterior margin with 2 indented points and medial indent. Pleotelson 0.5 times as long as anterior width, dorsal surface smooth, lateral margins posteriorly narrow, posterior margin broadly truncate, without median point.

Antennule more stout than antenna, comprised of 7 articles; peduncle articles 1 and 2 distinct and articulated; article 20.8 times as long as article 1 ; article 30.3 times as long as combined lengths of articles 1 and 2, 0.8 times as long as wide; flagellum
with 4 articles, extending to anterior of pereonite 1 . Antenna comprised of 9 articles. Antenna peduncle article 31.4 times as long as article 2, 1.1 times as long as wide; article 41.1 times as long as wide, 0.9 times as long as article 3; article 50.5 times as long as article 4, 0.8 times as long as wide. Antenna flagellum with 4 articles, last article terminating in no setae, extending to anterior margin of pereonite 1 . Anterior margin rounded, forming median point. Mandibular process ending in an acute incisor, with no simple setae, mandible palp article 2 with no distolateral setae, and article 3 with 7 serrate setae. Maxillule simple with 4 terminal robust setae. Maxilla mesial lobe partly fused to lateral lobe; lateral lobe with no simple setae, 6 recurved robust setae; mesial lobe with no simple setae, and 4 large recurved robust setae. Maxilliped weakly segmented, with lamellar oostegite lobe, palp article 2 with no simple setae, article 3 with 3 recurved robust setae, and no simple setae. Oostegites margin covered in numerous plumose setae, attached to pereopods $2-5$.

Pereopod 1 basis 1.6 times as long as greatest width; ischium 0.8 times as long as basis; merus proximal margin with bulbous protrusion; carpus with straight proximal margin; propodus 1.4 times as long as wide; dactylus slender, 1.1 as long as propodus, 2.5 times as long as basal width. Pereopod 2 propodus 1.5 as long as wide; dactylus 1.1 as long as propodus. Pereopods gradually increasing in size towards posterior and all without robust or simple setae. Pereopod 6 basis 1.6 times as long as greatest width, ischium 0.8 times as long as basis, propodus 1.5 as long as wide, dactylus 1.2 as long as propodus. Pereopod 7 basis 1.3 times as long as greatest width; ischium 0.9 as long as basis, without protrusions; merus proximal margin with slight bulbous protrusion, merus 0.3 as long as ischium, 0.5 times as long as wide; carpus 1 as long as ischium, without bulbous protrusion, 0.7 times as long as wide; propodus 2.1 as long as ischium, 1.4 times as long as wide; dactylus slender, 1.3 as long as propodus, 2.9 times as long as basal width.

Pleopods without setae, exopod larger than endopod. Pleopod 1 exopod 1.1 times as long as wide, lateral margin weakly convex, distally broadly rounded, mesial margin straight; endopod 1.4 times as long as wide, lateral margin convex, distally subtruncate, mesial margin straight; peduncle 3.3 times as wide as long, without retinaculae. Pleopods 2-5 similar to pleopod 1. Pleopods 3-5 endopods proximal borders do not extend below exopod to peduncle. Large medial lobes absent.

Uropod same length as pleotelson, peduncle 1 times longer than rami, peduncle lateral margin without setae; rami extending to pleotelson apex, marginal setae absent, apices narrowly rounded. Endopod apically slightly pointed, 4.1 times as long as greatest width, lateral margin straight, terminating without setae, mesial margin straight. Exopod extending to end of endopod, 3.3 times as long as greatest width, apically rounded, lateral margin weakly convex, terminating with no setae, mesial margin straight.

Male. Length 4-14 (8.2) mm, width $1-5$ (3.3) mm.
Males similar to females but much smaller. Body rectangular, 1.6 times as long as wide. Penis small, low tubercles. Pleopod 2 appendix masculina absent.

Etymology. A photograph by one of us (NJS) of this species in the mouth of a Diplodus sargus capensis from Tsitsikamma National park, posted on the internet in


Figure 8. Ceratothoa famosa sp. n. female holotype ( 28 mm ) (SAM A45939): A dorsal view B anterior view of pereonite 1 and cephalon $\mathbf{C}$ ventral view of cephalon $\mathbf{D}$ dorsal view of pleotelson $\mathbf{E}$ lateral view.


Figure 9. Ceratothoa famosa sp. n. female paratype ( 26 mm ) (SAM A45941): A antennule B antenna $\mathbf{C}$ mandible $\mathbf{D}$ tip of maxillule $\mathbf{E}$ maxillule $\mathbf{F}$ tip of maxilla $\mathbf{G}$ tip of maxilliped article $3 \mathbf{H}$ maxilla $\mathbf{I}$ maxilliped with oostegite $\mathbf{J}$ oostegites $\mathbf{K}$ uropod.


Figure 10. Ceratothoa famosa sp. n. female holotype ( 28 mm ) (SAM A45939): A pereopod 1 B pereopod 2 C pereopod $6 \mathbf{D}$ pereopod 7 .


Figure II. Ceratothoa famosa sp. n. female paratype ( 26 mm ) (SAM A45941): A dorsal pleopod 1 B dorsal pleopod $2 \mathbf{C}$ dorsal pleopod $3 \mathbf{D}$ dorsal pleopod 4 E dorsal pleopod $5 \mathbf{F}$ ventral pleopod 1 $\mathbf{G}$ ventral pleopod $2 \mathbf{H}$ ventral pleopod $3 \mathbf{I}$ ventral pleopod $4 \mathbf{J}$ ventral pleopod 5 .


Figure 12. Ceratothoa famosa sp. n. male paratype ( 12 mm ) (SAM A45941): A dorsal view B anterior view of pereonite 1 and cephalon $\mathbf{C}$ ventral view of cephalon $\mathbf{D}$ dorsal view of pleotelson $\mathbf{E}$ lateral view.


Figure 13. Ceratothoa famosa sp. n. male paratype ( 12 mm ) (SAM A45941): A antennule B antenna $\mathbf{C}$ maxillule $\mathbf{D}$ mandible $\mathbf{E}$ maxilla $\mathbf{F}$ maxilliped $\mathbf{G}$ uropod $\mathbf{H}$ pereopod 1 I pereopod 7 .


Figure 14. Ceratothoa famosa sp. n. male paratype ( 12 mm ) (SAM A45941): A dorsal pleopod 1 B dorsal pleopod $2 \mathbf{C}$ dorsal pleopod $3 \mathbf{D}$ dorsal pleopod $4 \mathbf{E}$ dorsal pleopod $5 \mathbf{F}$ ventral pleopod $1 \mathbf{G}$ ventral pleopod $2 \mathbf{H}$ ventral pleopod $3 \mathbf{I}$ ventral pleopod $4 \mathbf{J}$ ventral pleopod 5.

2004 has been used in many media reports worldwide, including magazines, children's books, documentaries, nature programmes, daily news reports, and even in a motion picture. The epithet is derived from famosus (Latin—famous) (Brown 1956).

Distribution. Known from off the southern coast of South Africa: Cape Agulhas; Knysna; Tsitsikamma; Swartkops River Estuary; Kenton-on-sea; Kleinemonde; Keiskamma River Mouth; Morgan Bay; Tshani; Grosvenor Point; and Transkei (between Goss Bay and Lupatana).

Hosts. Found on the tongue of Diplodus sargus capensis (Smith, 1844), Diplodus cervinus hottentotus (Smith, 1844) and Sparadon durbanensis (Castelnau, 1861).

Prevalence. 1/3 (33.3\%) of Diplodus cervinus hottentotus, 6/20 (30\%) of Diplodus sargus capensis and 6/33 (18.2\%) of Sparadon durbanensis infected from Tsitsikamma National Park; 26/366 (7.1\%) of Diplodus cervinus hottentotus, 78/1004 (7.8\%) of Diplodus sargus capensis and 11/100 (11\%) of Sparadon durbanensis from the SAIAB collections.

Remarks. Ceratothoa famosa sp. n. can be distinguished by the long rectangular body shape, pereonite 1 with a raised medial protrusion, a blunt rostrum, narrow antenna with antennule article 1 expanded, uropods which reach the posterior margin of the pleotelson, pereopods 1 and 2 with large bulbous protrusion on merus, narrow rami on uropods, and no appendix masculina on pleopod 2 of the male specimens.

Ceratothoa famosa sp. n . is similar to C. trigonocephala in having pereonites 1-4 almost subequal but has a more bluntly rounded anterior margin of the cephalon observed in C. imbricata as well as the two mediolateral concave indents in pleonite 5. Specific characters for C. famosa include an antennule with an enlarged first article; a medial protrusion on pereonite 1 creating a rounded elevation around the cephalon; and a rostral point which is folded over between the antennae. The anterolateral margins are close to the cephalon and are bluntly rounded extending just past the middle of the cephalon. The uropods are the same length as the pleotelson and the male specimens lack an appendix masculina on pleopod 2 as seen with C. oestroides (Risso, 1826), C. italica Schioedte \& Meinert, 1883, C. capri (Trilles, 1964c), C. gilberti (Richardson, 1904) and C. gaudichaudii. The pleopods do not have many folds or lobes but the pereopods have large carinae and extended protrusions on the merus of pereopods 1 and 2.

Other differences between C. famosa sp. n. and C. africanae sp. n. is the rostral point, which is blunt and ventrally directed and does not fold over in C. africanae.; C. famosa pereonite 7 overlaps pleonite 1 and the $\mathrm{P} 1-\mathrm{P} 4$ merus has a large bulbous protrusion which is smaller in P5-P7 (opposite in C. africanae sp. n.); and C. famosa. has pointed rather than rounded anterolateral margins on pereonite 1 as seen in C. africanae.

Miers (1876) commented that South African specimens in his possession from the Cape of Good Hope did not correspond to the specimens of C. imbricata in the British Museum and that there was a probability that the specimens were a distinct species. It is probable that all the records of C. imbricata (or C. banksii Leach, 1818) from South Africa are C. famosa sp. n.

## Excluded species

Two widely recorded species Ceratothoa imbricata (Fabricius, 1775) and Ceratothoa trigonocephala (Leach, 1818), both of which have long been considered to occur in South Africa (see Kensley 1978), are here excluded from the South African fauna. As there has been sustained confusion over the identity of these two species (see Miers 1884, Stebbing 1902, 1908, Nierstrasz 1915, Trilles 1973, Bruce et al. 2002), we present descriptions of the type material, and include differential remarks and brief comments on the distribution and host use for the species, based solely on those records we have been able to confirm. For a full synonymy and lists of all the host and locality records, see Trilles (1994) or Hadfield (2012).

## Ceratothoa imbricata (Fabricius, 1775)

http://species-id.net/wiki/Ceratothoa_imbricata
Figs 15-18, 21
Oniscus umbricatus Fabricius, 1775: 296.
Oniscus imbricatus.- Fabricius 1787: 241.
Cymothoa imbricata.- Fabricius 1793: 503; 1798: 304.
Cymothoa Banksii Leach, 1818: 353.
Ceratothoa Banksii.- Schioedte and Meinert 1883: 340-347, tab. XIV (Cym. XXI), Figs 6-21.
Ceratothoa imbricata.- Ellis 1981: 123.
Codonophilus imbricatus.- Hale 1926: 223-226, Figs 15-16; 1927: 315; 1929: 263-264, fig. 262; 1937: 19; 1940: 303.
Cymothoa banksii.- Ellis 1981: 124.

Material examined. Holotype of Ceratothoa imbricata. The Natural History Museum, London (BMNH 1979.403.1) - female ( $34 \mathrm{~mm} \mathrm{TL} ; 16 \mathrm{~mm} \mathrm{~W}$ ) collection of Sir Joseph Banks, Linnean Society, from New Zealand, coll. S.W.J. Banks, host unknown (Fabricius 1775). Noted: there is a hole in pereonite 4 and 5.

Holotype of Ceratothoa banksii. The Natural History Museum, London (BMNH 1979.402.1) - female ( 37 mm TL; 18 mm W), presented by Leach to the Museum of the Linnean Society, from New Zealand, White's MS Cat No. 222, Coll. W.E. Leach, host unknown.

Description of holotype. Body ovoid, 2.1 times as long as greatest width, dorsal surfaces slightly bumpy, widest at pereonite 5 , most narrow at pereonite 1 , lateral margins posteriorly ovate. Cephalon 0.7 times longer than wide, visible from dorsal view, triangular. Frontal margin rounded to form blunt rostrum. Eyes oval with distinct margins. Pereonite 1 with slight indentations, anterior border straight, anterolateral angle with distinct produced point extending to or beyond the eye margin, posterior
margins of pereonites smooth and slightly curved laterally. Pereonites $1-5$ increasing in length and width; 6-7 decreasing in length and width; 6 and 7 narrower. Pleon with pleonite 1 most narrow, visible in dorsal view; pleonites posterior margin smooth, mostly concave; posterolateral angles of pleonite 2 narrowly rounded, not posteriorly produced. Pleonites 3-5 similar in form to pleonite 2. Pleonite 5 with posterolateral angles free, not overlapped by lateral margins of pleonite 4, posterior margin produced medially. Pleotelson 2 times as long as anterior width, dorsal surface with lateral indent, lateral margins weakly convex, posterior margin rounded, without median point. Antennule more stout than antenna, comprised of 8 articles. Antenna comprised of 4 articles. Pereopod 1 basis 1.5 times as long as greatest width; ischium 0.8 times as long as basis; merus proximal margin with bulbous protrusion; carpus with straight proximal margin; propodus 1.5 times as long as wide; dactylus slender, 0.9 as long as propodus, 2.3 times as long as basal width. Pereopod 2 propodus 1.3 as long as wide; dactylus 0.6 as long as propodus. Pereopods 3 similar to pereopod 2. Pereopod 6 basis 1.4 times as long as greatest width, ischium 0.7 times as long as basis, propodus 1.5 as long as wide, dactylus 1 as long as propodus. Pereopod 7 basis 1.2 times as long as greatest width; ischium 0.7 as long as basis, without protrusions; merus proximal margin with slight bulbous protrusion, merus 0.4 as long as ischium, 0.6 times as long as wide; carpus 0.6 as long as ischium, without bulbous protrusion, 0.5 times as long as wide; propodus 0.6 as long as ischium, 1.4 times as long as wide; dactylus slender, 1.1 as long as propodus, 2.5 times as long as basal width. Uropod longer than the pleotelson, peduncle 0.7 times longer than rami, peduncle lateral margin without setae; rami extending beyond pleotelson, marginal setae absent, apices narrowly rounded.

Distribution. Australia (Schioedte and Meinert 1883, Miers 1884, Hale 1927, 1940), New Zealand (Fabricius 1775, 1793) and Indonesia (Schioedte and Meinert 1883).

Yu and Li (2003) included a figure of a specimen described as C. imbricata from Chinese waters; the figures show that the antenna bases do not touch, which excludes the species from Ceratothoa.

Hosts. From the mouth of a salmon-trout; from a Monacanthus sp. (Miers 1884); in the Australian jack mackerel, Trachurus declivis (Hale 1926, 1929); in snapper Chrysophrys auratus (previously Pagrosomus auratus), red gurnard (Chelidonichthys kumu), and mullet (Mugil sp.) (Hale 1926, 1929); in mouth of Girella tricuspidata (Hale 1926, 1929); trevally Psuedocaranx dentex (previously Caranx georgianus) (Hale 1926, 1929).

Schioedte and Meinert (1883) mention a fish they thought may be a "red hottentot (Sargi hottentotti Sm. ??)" collected from the Cape of Good Hope in South Africa. This could refer to the red roman fish, Chrysoblephus laticeps (see Kensley 1978), the Zebra (Diplodus cervinus hottentotus) or the Hottentot (Pachymetopon blochii) as "Sargi hottentotti" is not a valid taxonomic name and cannot be found in current fish database searches. No fresh material or museum material of these specimens from the red roman or those collected by Kensley (1978) could be found so these records are not accepted.


Figure I5. Ceratothoa imbricata (Fabricius, 1775), female holotype ( 34 mm ) (BMNH 1979.403.1): A dorsal view $\mathbf{B}$ antero-dorsal view of pereonite 1 and cephalon $\mathbf{C}$ dorsal view of pleotelson $\mathbf{D}$ lateral view.


Figure 16. Ceratothoa imbricata (Fabricius, 1775), female holotype ( 34 mm ) (BMNH 1979.403.1): A pereopod $1 \mathbf{B}$ pereopod $2 \mathbf{C}$ pereopod $6 \mathbf{D}$ pereopod 7 .


Figure 17. Ceratothoa imbricata (Fabricius, 1775), female ( 37 mm ), (BMNH 1979.402 .1 originally designated as holotype of Ceratothoa banksii Leach, 1818): A dorsal view B antero-dorsal view of pereonite 1 and cephalon $\mathbf{C}$ ventral view of cephalon $\mathbf{D}$ dorsal view of pleotelson $\mathbf{E}$ lateral view.


Figure 18. Ceratothoa imbricata (Fabricius, 1775), female ( 37 mm ), (BMNH 1979.402 .1 originally designated as holotype of Ceratothoa banksii Leach, 1818): A pereopod 1 B pereopod $2 \mathbf{C}$ pereopod 6 D pereopod 7 .

Remarks. Ceratothoa imbricata can be identified by a large pereonite 1 with anterolateral margins extending past the eyes; uropods as long or longer than the pleotelson margin; merus with bulbous protrusion; a blunt rostrum; and body widest at pereonite 5 .

It is apparent that over the years there have been many misidentifications of $C$. imbricata, C. banksii and C. trigonocephala, with these names being widely misapplied. The description of C. banksii from New Zealand, given by Miers (1876) can also be applied to the small Australian C. imbricata specimens of Miers (1884), with only some slight variations in eyes, smaller anterolateral extensions on pereonite, 1 and a slightly arched pleotelson posterior margin. The original description of C. banksii by Leach (1818) also described the pleotelson as "nearly straight" but according to Miers (1884), desiccation had caused the specimen to roll slightly. Many authors agreed with the synonymy of Ceratothoa banksii with C. imbricata including Stebbing (1893), Nierstrasz (1915) and Trilles (1973) and we maintain this synonymy, however this needs further investigation, especially when fresh material becomes available.

Trilles (1994) placed Hale's (1926, 1927, 1929, 1940) records of C. imbricata into synonymy with C. trigonocephala. After reviewing Hale's (1926) figures, we conclude that his original identification of C. imbricata is correct.

No South African specimens were found, fresh or from museum collections, that could be identified as C. imbricata, and the species is here excluded from the South Africa fauna.

## Ceratothoa trigonocephala (Leach, 1818)

http://species-id.net/wiki/Ceratothoa_trigonocephala
Figs 19-21
Cymothoa trigonocephala Leach, 1818: 353; Guérin-Méneville and Cuvier 1829-1843: 26, pl. 29, fig. 2; Milne Edwards 1840: 272-273; Ellis 1981: 124.
Ceratothoa trigonocephala.- Schioedte and Meinert 1883: 358-364, tab. XVI (Cym. XXIII) Figs 1-7.

Material examined. Lectotype [here designated]: The Natural History Museum, London (NHMUK 2013.1013) - female specimen ( 42 mm TL ) collected by W.E. Leach, White's MS Cat no. $404 \mathrm{a}, \mathrm{b}$, host and locality unknown. Also noted: the female drawn was very squashed and missing pereonite 1 .

Paralectotype. The Natural History Museum, London (BMNH 1979.404.2) - female specimen ( 17 mm TL without cephalon) collected by W.E. Leach, White's MS Cat no. $404 \mathrm{a}, \mathrm{b}$, host and locality unknown. Also noted: damaged female, missing the cephalon and oostegites, with dissected uropods.

Description of lectotype. Body margins sub-parallel, 2.4 times as long as greatest width, dorsal surfaces smooth and polished in appearance, widest at pereonite 5 and pereonite 6 , most narrow at pereonite 1 , lateral margins subparallel. Cephalon 0.6 times longer than wide, visible from dorsal view, triangular. Frontal margin rounded
to form blunt rostrum. Eyes not visible. Pereonite 1 with slight indentations, anterior border slightly indented, anterolateral angle with distinct anterior projection, posterior margins of pereonites smooth and straight. Pereonites $1-5$ increasing in length and width; 6-7 decreasing in length and width; 6 and 7 narrower. Pleon with pleonite 1 most narrow, visible in dorsal view; pleonites posterior margin smooth, mostly concave; posterolateral angles of pleonite 2 rounded, not posteriorly produced. Pleonites $3-5$ similar in form to pleonite 2 . Pleonite 5 with posterolateral angles free, not overlapped by lateral margins of pleonite 4, posterior margin produced medially. Pleotelson 0.5 times as long as anterior width, dorsal surface with lateral indent, lateral margins weakly convex, posterior margin sub-truncate, without median point. Antennule more stout than antenna, comprised of 7 articles. Antenna comprised of 7 articles. Pereopod 1 basis 1.4 times as long as greatest width; ischium 0.8 times as long as basis; merus proximal margin without bulbous protrusion; carpus with rounded proximal margin; propodus 1.4 times as long as wide; dactylus slender, 1.2 as long as propodus, 2.3 times as long as basal width. Pereopod 2 propodus 1.4 as long as wide; dactylus 1.1 as long as propodus. Pereopods 3 similar to pereopod 2. Pereopod 6 basis 1.5 times as long as greatest width, ischium 0.8 times as long as basis, propodus 1.5 as long as wide, dactylus 1.1 as long as propodus. Pereopod 7 basis 1.4 times as long as greatest width; ischium 0.8 as long as basis, without protrusions; merus proximal margin with slight bulbous protrusion, merus 0.4 as long as ischium, 0.7 times as long as wide; carpus 0.3 as long as ischium, without bulbous protrusion, 0.7 times as long as wide; propodus 0.6 as long as ischium, 1.6 times as long as wide; dactylus slender, 1.2 as long as propodus, 2.5 times as long as basal width. Uropod more than half the length of pleotelson, peduncle 0.7 times longer than rami, peduncle lateral margin without setae; rami not extending beyond pleotelson, marginal setae absent, apices narrowly rounded.

Distribution. Predominately the Indo-Pacific region: Australia; Vanuatu; and Indonesia (Schioedte and Meinert 1883), but given the uncertainty over the identity of these records the distribution remains entirely uncertain.

Hosts. There are currently no confirmed hosts for this species.
Remarks. Ceratothoa trigonocephala has a triangular cephalon, for which it is named, and arched carinae on the last pair of pereopods. It is identified by the subequal pereonites $1-4$; mid-dorsal protrusion on pereonite 1 ; short and bluntly rounded anterolateral margins of pereonite 1 ; and uropods which do not extend past the pleotelson posterior margin. The type locality and host for C. trigonocephala were not mentioned in the original work by Leach (1818).

Previously, Filhol (1885) noted that C. trigonocephala, C. buttoni Filhol, 1885 and C. novaezelandiae Filhol, 1885 were three separate species based on small morphological differences. Some of these differences included the shape of the antennae; the shape and dimensions of the pereonites; and pigmentation. All three of these species were later combined into one as C. trigonocephala, with the differences recognised as intraspecific and not interspecific (Trilles 1972). After reviewing the drawings, C. huttoni was found to not resemble the C. trigonocephala holotype and the identity of C. novaezelandiae could not be confirmed and thus these synonymies are not upheld here.


Figure 19. Ceratothoa trigonocephala (Leach, 1818), female lectotype ( 42 mm ) (NHMUK 2013.1013): A dorsal view $\mathbf{B}$ antero-dorsal view of pereonite 1 and cephalon $\mathbf{C}$ dorsal view of pleotelson $\mathbf{D}$ lateral view.


Figure 20. Ceratothoa trigonocephala (Leach, 1818), female lectotype ( 42 mm ) (NHMUK 2013.1013): A pereopod $1 \mathbf{B}$ pereopod $2 \mathbf{C}$ pereopod $6 \mathbf{D}$ pereopod 7 .


Figure 21. Photographs of the Ceratothoa specimens studied: A lateral view of C. africanae sp. n. (SAM A45938) B dorsal view of C. africanae sp. n. (SAM A45938) C lateral view of C. famosa sp. n. (SAM A45941) D dorsal view of C. famosa sp. n. (SAM A45941) E dorsal view of C. imbricata (Fabricius, 1775) (BMNH 1979.403.1) F dorsal view of C. trigonocephala (Leach, 1818) (NHMUK 2013.1013).

Ceratothoa trigonocephala has often been confused with C. imbricata and a complete redescription and species clarification on these two species was needed. Differences between C. imbricata and C. trigonocephala, based on description of the type specimens include: Ceratothoa imbricata pereonite 1 is larger than pereonites $2-4$ while in C. trigonocephala these four pereonites are subequal; the posterior margin of pereonite 1 is curved in C. imbricata and straight in C. trigonocephala; C. imbricata had a bulbous protrusion on the merus of pereopod 1 which is absent in C. trigonocephala; and the uropods of $C$. trigonocephala are shorter than the pleotelson but are longer in C. imbricata. Furthermore, C. imbricata has a more narrow and produced anterolateral angles on pereonite 1; a more rounded anterior margin on the cephalon; longer uropods which extend to or past the posterior margin of the pleotelson; and pereonite 1 is longer than pereonites 2-4 which are almost subequal in C. trigonocephala.

Although this species had been recorded from South Africa (Kensley 1978, 2001), no South African specimens were found during the present study that could be identified as C. trigonocephala, and the species is here excluded from the South Africa fauna.

## Conclusion

We regard Ceratothoa imbricata and C. trigonocephala as valid and distinct species despite the historical confusion over their respective identities. When comparing the holotype of C. banksii to C. imbricata, a number of similarities and differences could be seen but without other new material these differences seemed insufficient to remove the synonymy at present.

Records of C. imbricata and C. trigonocephala without figures or mention of museum material are impossible to verify. The synonymy presented here includes only those records that we can confirm against our redescription of the type material.

Although valid species, C. imbricata and C. trigonocephala do not occur in South Africa. These misidentifications were most probably referring to one of the two new species, C. africanae sp. n. or C. famosa sp. n.

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# Petrocephalus boboto and Petrocephalus arnegardi, two new species of African electric fish (Osteoglossomorpha, Mormyridae) from the Congo River basin 

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#### Abstract

A specimen of the African weakly electric fish genus Petrocephalus (Osteoglossomorpha, Mormyridae) collected in the Congo River at Yangambi, Orientale Province, Democratic Republic of Congo, is described as a new species. Petrocephalus boboto sp. n. can be distinguished from other Central African species of Petrocephalus by a combination of the following characteristics: three distinct black spots on the body, one at the origin of the pectoral fin, one at the origin of the caudal fin and one below the anterior base of the dorsal fin; Nakenrosette and Khelrosette electroreceptor clusters distinct on head but Augenrosette cluster reduced in size; 23 branched dorsal rays, 34 branched anal rays, and electric organ discharge waveform triphasic. Petrocephalus boboto sp. n. most closely resembles the holotype of Petrocephalus binotatus but is easily distinguished from it by its smaller mouth. A comparative molecular analysis including 21 other Petrocephalus species shows Petrocephalus boboto sp. n. to be genetically distinctive and to represent a deep lineage in the genus. Two specimens of Petrocephalus collected at Yangambi are morphologically similar and genetically closely related to specimens previously assigned to Petrocephalus binotatus, collected in the northwestern Congo River basin within Odzala-Kokua National Park, Republic of the Congo. This prompts us to formally describe a new species from these collections, Petrocephalus arnegardi sp. n., that, although similar to the holotype of Petrocephalus binotatus, can be distinguished from it by its smaller mouth and shorter interorbital width.


#### Abstract

Résumé Un spécimen de poissons africains à faible décharge électrique du genre Petrocephalus (Osteoglossomorpha, Mormyridae) collecté dans le fleuve Congo à Yangambi, Province Orientale, République Démocratique du Congo, est décrit comme une nouvelle espèce. Petrocephalus boboto sp. n. peut-être distinguée des autres espèces de Petrocephalus d'Afrique Centrale par la présence de trois distinctes taches noires, l'une à la base des nageoires pectorales, la seconde à la base de la nageoire caudale et la troisième sur chacun des flancs, juste en dessous des premiers rayons de la nageoire dorsale; présence bien visible de deux rosettes électroréceptives sur la tête (Nakenrosette et Khelrosette), la troisième rosette (Augenrosette) est de taille réduite; 23 rayons branchus à la nageoire dorsale et 34 rayons à la nageoire anale; et décharge électrique triphasique. Petrocephalus boboto sp. n. ressemble le plus à l'holotype de Petrocephalus binotatus mais s'en distingue par une bouche nettement plus petite. Une analyse génétique confirme la distinction de Petrocephalus boboto sp. n. parmi un échantillon de 21 espèces. Cette analyse montre également que cette nouvelle espèce représente une lignée distincte de Petrocephalus. Deux autres spécimens de Petrocephalus collectés à Yangambi sont morphologiquement et génétiquement proches de spécimens auparavant identifiés comme appartenant à Petrocephalus binotatus, collectés dans le Parc National d'Odzala-Kokua, bassin du Congo, République du Congo. Ceci nous amène à les décrire comme une nouvelle espèce, Petrocephalus arnegardi sp. n. qui peut-être distinguée de l'holotype de Petrocephalus binotatus par une bouche plus petite et un espace inter-orbitaire plus court.


## Keywords

Biodiversity, weakly electric fish, Petrocephalinae, integrative taxonomy, phylogeny, cytochrome $b$, electric organ discharge

## Introduction

The monophyletic African weakly electric fishes superfamily Mormyroidea (Teleostei, Osteoglossomorpha) contains two families, the Gymnarchidae and Mormyridae, and 212 species (Eschmeyer and Fong 2014). All mormyroids generate and sense weak electric discharges for the purpose of intraspecific electrocommunication and spatial electrolocation using complex electrogenic and electroreceptive organ systems (Moller 1995, Turner et al. 1999, Bullock et al. 2005). While mormyroids are immediately identifiable by their distinctive appearance, considerable morphological, electrophysiological and behavioral diversity has evolved within the group (Arnegard et al. 2010b, Carlson et al. 2011, Rabosky et al. 2013). These differences lead to the recognition of three main lineages: the monotypic Gymnarchidae and the two reciprocally monophyletic mormyrid subfamilies Mormyrinae and Petrocephalinae with 168 and 43 valid species, respectively (Taverne 1972, Sullivan et al. 2000, Eschmeyer and Fong 2014).

Recent comparative studies have revealed significant electrophysiological differences between Mormyrinae and Petrocephalinae (Lavoué et al. 2008, Carlson and Arnegard 2011, Carlson et al. 2011). In particular, Carlson et al. (2011) suggested that differences among lineages in central and peripheral electrosensory anatomies are correlated with higher electric organ discharge (EOD) waveform diversity and greater species richness in Mormyrinae relative to the Petrocephalinae. While Mormyrinae are clearly more speciose than Petrocephalinae, true species diversity is clearly underestimated in both groups. Despite often subtle morphological differentiation among
species of Petrocephalus, the sole petrocephalin genus, the pace of new species discovery and description has been rapid in recent years, with about one third of Petrocephalus diversity having been described in the 21th century (Lavoué et al. 2004, 2010, Lavoué 2011, 2012, Kramer et al. 2012).

Petrocephalus currently includes 43 valid species (Eschmeyer and Fong 2014), not including Petrocephalus balteatus (for justification, see Daget 2000), which are widely distributed in tropical and subtropical African freshwaters. They are small fishes predominantly found in riverine systems where they are mostly active at dusk. Several morphological synapomorphies support the monophyly of Petrocephalus (Taverne 1969, Taverne 1972), as do molecular data (Sullivan et al. 2000).

Central African Petrocephalus are particularly diverse and abundant throughout the large Congo basin with 18 species (Lavoué et al. 2010, Lavoué 2012). Here, we first describe a new species of Petrocephalus from the main channel of the Congo River at Yangambi, Central Congo basin. Based on our long-term work on Petrocephalus, we believe this new species is rare, as we have identified only one specimen after examination of hundreds of Central African Petrocephalus specimens. From the same locality, we examined two specimens of Petrocephalus that share more similarities to the specimens earlier identified as Petrocephalus binotatus from Odzala-Kokua (Lavoué et al. 2010) than to the holotype of $P$. binotatus, despite the greater proximity of Yangambi to the type locality of $P$. binotatus (Ikengo), than to Odzala-Kokua (Fig. 1). This led us to reevaluate this identification and describe the Odzala-Kokua and Yangambi specimens as new.

## Materials and methods

Petrocephalus specimens newly examined in this study were collected in September 2010 from the Congo River at Yangambi, Orientale Province, Democratic Republic of Congo (Fig. 1). Comparative material including type specimens from all valid species from the Congo River basin and the Lower Guinea province is listed in Lavoué et al. $(2004,2010)$ and Lavoué $(2012)$. About half of the nucleotidic sequences of the cytochrome $b$ gene (about 600 base pairs from the $3^{\prime}$ end) were determined for three specimens of Petrocephalus collected at Yangambi.

In the field, we recorded EODs from two of these three specimens of Petrocephalus. Each individual was first transferred to a plastic tank (ca. 80 cm long* 30 cm wide* 30 cm deep) filled with water from the capture location. EODs were recorded with silver/silverchloride electrodes connected to an Edirol FA-66 analog-to-digital Firewire audio interface (Roland Corporation). Signals were visualized and stored in SignalScope 2.0 virtual oscilloscope software (Faber Acoustical) such that positivity at the fish's head was always recorded in the upward direction. Water temperature of each recording was noted. Fish were euthanized with an overdose of MS222, photographed, tissued, tagged with a unique specimen number, fixed in buffered $10 \%$ formalin and later transferred to $70 \%$ ethanol.

Total peak-to-peak amplitudes of all EODs were normalized to a value of one. We did not correct the time base of EODs to a uniform temperature as this procedure pro-


Figure I. Hydrographic system of the Congo River basin and the type localities of Petrocephalus boboto sp. n. (black-filled star) at Yangambi, Democratic Republic of Congo, Petrocephalus arnegardi sp. n. (grayfilled star) at Odzala-Kokua National Park, Republic of the Congo and Petrocephalus binotatus (whitefilled star) at Ikengo, near Mbandaka, Democratic Republic of Congo.
duces only minimal changes. Seven EOD measures were taken as described in Lavoué et al. (2008): relative height of peak 1, relative height of peak 2 , relative height of peak 3 (when present), duration of peak 1, duration of peak 2, duration of peak 3 (when present), total EOD duration. EOD recordings have been archived in the Macaulay Library at the Cornell Lab of Ornithology (CUML accession numbers provided in Suppl. material 1).

Mature male specimens were identified by the presence of an anal fin notch (Pezzanite and Moller 1998). Methods for making counts and measurements and their
abbreviations follow those given by Boden et al. (1997), except for body height and standard length that are modified as in Lavoué et al. (2004). Furthermore, the snout length and the mouth position are the nearest distances, parallel to the body axis, from the perpendicular line through the tip of the snout to the anterior border of the eye and the corner of the mouth, respectively.

We examined the specimens for the presence of each of the three distinct clusters of Knollenorgan-type electroreceptors on the head of Petrocephalus, as described in Harder $(1968,2000)$. The "Augenrosettes" are directly above the anterior half of the eye, the "Nackenrosettes" are dorsally situated on each side of the nape, slightly anterior to the opercular openings and the "Kehlrosettes" are situated anterior to and extending somewhat below the insertion of the pectoral fins.

DNA was extracted from fin clips preserved in $90 \%$ ethanol. PCR amplification and sequencing of the partial cytochrome $b$ gene were as described by Sullivan et al. (2000) using the following Petrocephalus-specific primers: L15213_MOR (5'CTA ACC CGA TTC TTT GCC TTC CAC TTC CT-3’) and H15913_MOR (5'-TCG ATC TCC GGA TTA CAA GAC CG-3'). Cytochrome $b$ sequences generated in this study are available in GenBank under accession numbers KF181719 to KF181721. The three new partial cytochrome $b$ sequences were added to the dataset published in Lavoué (2012) from which individuals uninformative for interspecific comparisons have been removed. Following the conclusions of Kramer et al. (2012) who resurrected several species previously synonymized with Petrocephalus catostoma, we assigned revised names to two specimens (and their corresponding cytochrome $b$ sequences) treated in Lavoué (2012): one specimen of P. catostoma from the Rufiji River is renamed Petrocephalus steindachneri and one specimen of P. catostoma from the Wami River is renamed Petrocephalus stuhlmanni. The alignment does not require any indels and missing data were coded with "-". The final alignment comprises 1140 nucleotidic positions. The maximum likelihood phylogenetic tree was calculated under the general time reversible model of nucleotide substitution with rate heterogeneity following a discrete gamma distribution $(\mathrm{GTR}+\Gamma)$, using the software RAxML-VI-HPC (Stamatakis 2006) and its graphical interface raxmlGUI (Silvestro and Michalak 2012).

## Taxonomy

## Petrocephalus boboto sp. n .

http://zoobank.org/A0EE9192-B7D4-4565-A598-E251D86AD5D4
http://species-id.net/wiki/Petrocephalus_boboto
Figs 2 and 3; Table 1

Holotype. CUMV 96774, tag no. JPS-10-426, 56.9 mm SL, sex undetermined, Orientale Province, Democratic Republic of Congo: Congo River at Yangambi, $0.76^{\circ} \mathrm{N}$, $24.24{ }^{\circ}$ E, Lavoué et al., 10 September 2010.

Diagnosis. Petrocephalus boboto sp. n . is distinguished from all other Petrocephalus species of Central Africa (i.e., Lower Guinea and Congo provinces) by the following combination of characteristics: three distinctive bilateral black spots on body, one at origin of the pectoral fin, one at origin of caudal fin and one on flank just below anterior part of dorsal fin; two distinct electoreceptive rosettes in head (i.e., Nakenrosette and Khelrosette), the third rosette (Augenrosette) reduced in size to few knollenorgan pores; 23 branched rays in dorsal fin and 34 branched rays in anal fin; triphasic EOD waveform of normal polarity (i.e. first phase head-positive).

Description. Morphometric ratios and meristic data for holotype presented in Table 1. Standard length $=56.9 \mathrm{~mm}$. Sex undetermined: specimen lacks anal fin "notch" present in mature males, but may be juvenile. Body ovoid, 2.7 times longer than high and laterally compressed. Head length 3.3 times in standard length. Snout short (HL/ $\mathrm{SNL}=6.6$ ) and round. Mouth small (HL/MW = 4.4), subterminal, opening under anterior half of eye. Teeth small and bicuspid, 13 in single row in upper jaw, 24 in single row in lower jaw. Nostrils closely set (distance between nostrils $=0.4 \mathrm{~mm}$ ) with the posterior one very close to eye (distance between posterior nostril and eye $=0.1 \mathrm{~mm}$ ). Dorsal and anal fins originating in posterior half of body ( $\mathrm{SL} / \mathrm{PDD}=1.6$ and $\mathrm{SL} / \mathrm{PAD}=1.8$ ). Predorsal distance slightly greater than pre-anal distance ( $\mathrm{PDD} / \mathrm{PAD}=1.1$ ). Dorsal fin with 23 branched rays. Anal fin with 34 branched rays. Pectoral fins with 9 rays. Pelvic fin with 6 rays. Distance between pelvic fin and anal fin $=10.1 \mathrm{~mm}$. Distance between pectoral fin and anal fin $=16.4 \mathrm{~mm}$. Scales cover body, except for head. Lateral line visible and complete with 34 pored scales along its length. Ten scales around caudal peduncle. Ten scales between anterior base of anal fin and lateral line. Caudal peduncle narrow (CPL/CPD = 1.6). Thick skin on head. Knollenorgans visible, clustered into two distinct rosettes, Nackenrosette and Kehlrosettes as described in Harder (1968). Knollenorgan pores in front of eye not highly condensed in the form of a discernable Augenrosette.

Electric organ discharge (Fig. 2A). Short triphasic EOD waveform, first phase head-positive. Relative height of peak $1=0.241$, relative height of peak $2=-0.759$, relative height of peak $3=0.053$, duration of peak $1=0.137 \mathrm{msec}$, duration of peak $2=0.041 \mathrm{msec}$, duration of peak $3=0.079 \mathrm{msec}$, total EOD duration $=0.257 \mathrm{msec}$. Based on characteristics of the EOD, the electrocytes are assumed to have non-penetrating stalks and to be innervated posteriorly (Sullivan et al. 2000).

Live coloration (Fig. 3). Body background color uniformly gray/silver with metallic reflection on the flanks and head. Light melanophores densely and evenly distributed on body, slightly larger on head. Three distinct black marks on each side of the body, one at the base of the pectoral fins, one at the base of the caudal fin and one subdorsal, below the first anterior rays of the dorsal fin. All fins hyaline.

Preserved coloration (Fig. 3). Background beige with dorsum slightly darker. Numerous light melanophores visible all over body. Thick skin on head and fins opaque. Three distinct melanin marks on side of body distinctly visible.

Distribution (Fig. 1). Holotype (and only specimen known) of Petrocephalus boboto sp. n. collected from the main channel of the Congo River, at Yangambi, Orientale Province, Democratic Republic of Congo.


Figure 2. A EOD waveform of the holotype of Petrocephalus boboto sp. n. from Yangambi (CUMV 96774) B EOD waveform of the specimen JPS-511 of Petrocephalus arnegardi from Yangambi (BMNH 2013.8.29.125). Waveform plotted with head-positivity upwards.

Phylogenetic affinities (Fig. 4). Petrocephalus boboto sp. n. represents a deep lineage within the genus Petrocephalus not closely related to any of the four described species exhibiting a similar pattern of markings: Petrocephalus zakoni, Petrocephalus odzalaensis, Petrocephalus balayi and Petrocephalus arnegardi sp. n. However, it is worth noting that Petrocephalus binotatus, the fifth species with a potentially similar pigmentation, is not included in this tree and the phylogenetic position of this species is unresolved.

Etymology. The name boboto is a word in Lingala, the language spoken at the type locality of Petrocephalus boboto sp. n., meaning "peace," alluding to the right of all people of D.R. Congo to live in peace and safety.

Comparison. The distinctive pattern of melanin markings of Petrocephalus boboto sp. n., having three dark spots-one at the origin of the pectoral fin, a second at the origin of the caudal fin, and a third on the flank just below the anterior base of the dorsal fin-distinguishes this species from most of its congeners. Only five other species of Petrocephalus similarly possess three black marks in these locations: P. odzalaensis, $P$. balayi, $P$. zakoni, $P$. binotatus and $P$. arnegardi sp. n. (the comparison with $P$. arnegardi $\mathrm{sp} . \mathrm{n}$. is provided under the description of this species). Petrocephalus boboto sp. n. can immediately be distinguished from $P$. zakoni by the presence of two of the three electroreceptor rosettes on the head that are typical to Petrocephalus (versus absence of all three in $P$. zakoni) and a higher number of anal fin rays (33 in $P$. boboto sp. n . versus
Table I. Principal morphometric ratios and meristic counts for the holotype (CUMV 96774) of Petrocephalus boboto sp. n. from Yangambi, the holotype (MRAC 15191) of Petrocephalus binotatus from Ikengo, the holotype (CUMV 88074) and the 17 paratypes (CUMV 88076, 88079, 88041, 87838, 88063, 87785, 88052, 88053, $92390,87830,88080,88123,88064,88065,88031$ and 88032 ) of Petrocephalus arnegardi sp. n., all from Odzala-Kokua National Park, and two other specimens (BMNH 2013.8.29.34 and 2013.8.29.125) of Petrocephalus arnegardi sp. $n$. from Yangambi. Abbreviations: $u=s e x$ undetermined; $m=$ sexually mature male; Min-Max= minimum-maximum; stdev= standard deviation. "*" indicates data from Pellegrin (1924).

|  | P. boboto sp. n . <br> Holotype (u) from Yangambi | P. binotatus <br> Holotype $(\mathrm{m})$ from <br> Ikengo | Holotype (m) from Odzala | P. arnegardi sp. n. |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  |  | Paratypes from Odzala |  | Two non-types ( $\mathrm{u}, \mathrm{m}$ ) from Yangambi |
|  |  |  |  | Min-Max | Mean (stdev) |  |
| Standard length (mm) | 56.9 | 83.2 | 72.6 | 62.8-90.1 | 72.1 | 48.9, 76.0 |
| Head length (mm) | 17.4 | 23.1 | 21.6 | 17.3-24.6 | 20.3 | 12.3, 20.2 |
| Ratio of standard length (SL): |  |  |  |  |  |  |
| SL/body height (H) | 2.7 | 2.4 | 2.5 | 2.3-2.8 | 2.6 (0.2) | 2.8, 2.6 |
| SL/head length (HL) | 3.3 | 3.6 | 3.4 | 3.4-3.9 | 3.6 (0.2) | 4.0, 3.8 |
| SL/pre-dorsal distance (PDD) | 1.6 | 1.6 | 1.5 | 1.4-1.6 | 1.5 (0.0) | 1.6, 1.6 |
| SL/pre-anal distance (PAD) | 1.8 | 1.7 | 1.6 | 1.6-1.7 | 1.7 (0.0) | 1.8, 1.7 |
| SL/dorsal fin length (DFL) | 5.1 | 4.5 | 4.9 | 4.2-5.4 | 5.0 (0.3) | 5.0, 4.9 |
| SL/anal fin length (AFL) | 3.0 | 2.8 | 3.1 | 2.8-3.3 | 3.0 (0.2) | 3.2, 3.1 |
| SL/caudal peduncle length (CPL) | 6.3 | 7.0 | 6.4 | 5.7-7.5 | 6.7 (0.5) | 5.8, 7.6 |
| SL/mouth width (MW) | 14.2 | 11.6 | 15.1 | 14.7-18.4 | 16.4 (1.1) | 18.0, 15.8 |
| Ratio of head length (HL): |  |  |  |  |  |  |
| HL/snout length (SNL) | 6.6 | 5.5 | 8.3 | 6.5-9.3 | 7.5 (0.8) | 5.1, 5.8 |
| HL/mouth width (MW) | 4.4 | 3.2 | 4.5 | 4.1-5.0 | 4.6 (0.2) | 4.5, 4.2 |
| HL/eye diameter (ED) | 4.4 | 3.6 | 4.1 | 3.5-4.4 | 4.0 (0.3) | 4.2, 4.7 |
| HL/interorbital width (IOW) | 3.7 | 2.3 | 3.3 | 2.6-3.9 | 3.2 (0.3) | 2.9, 2.6 |
| HL/head width (HW) | 2.4 | 1.9 | 2.2 | 1.8-2.2 | 2.1 (0.1) | 1.9, 1.9 |
| HL/mouth position (MP) | 4.4 | 3.9 | 5.5 | 4.2-5.6 | 4.8 (0.4) | 4.4, 4.4 |
| Ratio of caudal peduncle length (CPL): |  |  |  |  |  |  |
| CPL/caudal peduncle depth (CPD) | 1.6 | 2.3 | 2.2 | 1.8-2.5 | 2.2 (0.2) | 2.6, 2.0 |


|  | P. boboto sp. n . <br> Holotype (u) from Yangambi | P. binotatus <br> Holotype (m) from <br> Ikengo | Holotype (m) from Odzala | P. arnegardi sp. n. |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  |  | Paratypes from Odzala |  | Two non-types ( $u, m$ ) from Yangambi |
|  |  |  |  | Min-Max | Mean (stdev) |  |
| Meristic counts: |  |  |  |  |  |  |
| Dorsal fin branched rays (DR) | 23 | 24 | 20 | 20-22 | 21 (1) | 20,22 |
| Anal fin branched rays (AR) | 34 | 33 | 32 | 31-34 | 32 (1) | 30, 32 |
| Number of lateral line scales (SLL) | 34 | 37 | 37 | 34-38 | 36 (1) | 36,36 |
| Number of scale rows between anterior base of anal fin and lateral line (SDL) | 10 | 11 | 12 | 10-13 | 11 (1) | 10, 10 |
| Number of teeth in upper jaw (TUJ) | 13 | 15* | 8 | 9-16 | 10 (1) | 9, 11 |
| Number of teeth in lower jaw (TLJ) | 24 | $24^{*}$ | 20 | 20-24 | 21 (1) | 25,21 |



Figure 3. Photographs of the holotype of Petrocephalus boboto sp. n. from Yangambi, Congo River, Democratic Republic of Congo. Top photograph, the holotype specimen ( 56.9 mm standard length) before preservation showing appearance in life (CUMV 96774); middle and bottom photographs represent the left and right sides of the preserved holotype. Scale bar equals one centimeter.


Figure 4. Phylogeny of Petrocephalus ( 23 species, 52 specimens) estimated by maximum likelihood analysis of cytochrome $b$ nucleotide sequences. Mormyrin Gnathonemus petersii, Mormyrops nigricans and Myomyrus macrops were used as outgroups to root the tree. Numbers at internal branches are bootstrap proportions (in \%) shown only for interspecific relationships and when they exceed $50 \%$. Black-filled vertical bars to the right of the tree indicate the river basin origins of the specimens. The scale bar corresponds to 0.04 substitutions per site. Petrocephalus boboto sp. n. and Petrocephalus arnegardi sp. n. are highlighted in gray.
a maximum of 28 in P. zakoni). Petrocephalus boboto sp. n. can be distinguished from $P$. balayi and $P$. odzalaensis by its higher number of anal fin rays (33 in P. boboto sp. n. versus a maximum of 27 and 20 in $P$. balayi and $P$. odzalaensis, respectively). Petrocephalus balayi also has a proportionally larger mouth (HL/MW $=2.7-3.9$, mean= 3.3 versus 4.4 in P. boboto sp. n.). Petrocephalus odzalaensis has a proportionally wider head $(\mathrm{HL} / \mathrm{IOW}=2.5-3.0$, mean $=2.8$ and $\mathrm{HL} / \mathrm{HW}=1.7-1.8$, mean $=1.7$ versus 3.7 and 2.7 in $P$. boboto sp. n., respectively) and its body background color is more pinkish rather than gray/silver in P. boboto sp. n. Petrocephalus boboto sp. n. is distinguished from the holotype of $P$. binotatus by a smaller mouth (SL/MW $=14.2$ and HL/MW $=4.4$ versus 11.6 and 3.2 , respectively, in the holotype of $P$. binotatus), a smaller eye ( $\mathrm{HL} / \mathrm{ED}=4.4$ versus 3.6 in the holotype of $P$. binotatus), and a smaller interorbital distance (HL/IOW $=3.7$ versus 2.3 in the holotype of $P$. binotatus).

The holotype of $P$. boboto $\mathrm{sp} . \mathrm{n}$. is smaller than the holotype of $P$. binotatus. The differences between $P$. boboto sp. n. and $P$. binotatus are unlikely to be the result of growth allometry since allometric differences have only been observed with respect to the number of teeth in some species of Petrocephalus (Bigorne and Paugy 1991), a character that does not differentiate these two species. Although we are confident of the specific distinction between $P$. boboto sp. n. and $P$. binotatus, more specimens are needed to better characterize the morphological, molecular and electrophysiological differences between them.

## Petrocephalus arnegardi sp. n.

http://zoobank.org/23BDE2CA-426C-469F-B124-07E73C22EE82
http://species-id.net/wiki/Petrocephalus_arnegardi
Figs 2 and 5; Table 1
Petrocephalus sp. 1, OTU 1 in Lavoué et al. (2008).
Petrocephalus binotatus in Lavoué et al. (2010), Lavoué (2011, 2012), Carlson et al. (2011).

Holotype. CUMV 88074, tag no. $5074,72.6 \mathrm{~mm}$ SL, male, Republic of the Congo: Cuvette Ouest, Congo River basin, Pandaka River, Odzala-Kokua National Park, $0.62^{\circ}$ N, $14.92^{\circ}$ E, Friel et al., August 2002.

Paratypes (17). Republic of the Congo: Cuvette-Ouest: Congo River basin: CUMV 88076, tag no. $5076,69.6 \mathrm{~mm}$ SL, male, same data as holotype; CUMV 88041, tag no. 5120 , 85.1 mm SL , sex undetermined, same data as holotype; CUMV 88080, tag no. 5083, 72.0 mm SL, male, same data as holotype; CUMV 88032, tag no. 5101, 73.0 mm SL, male, same data as holotype; CUMV 87785, tag no. 5097, 71.3 mm SL, sex undetermined, same data as holotype; CUMV 88031, tag no. 5100, 73.7 mm SL, sex undetermined, same data as holotype; CUMV 88079, tag no. 5082, 74.8 mm SL, sex undetermined, Lékénie River at Mboko landing, Odzala-Kokua National Park, $0.62^{\circ} \mathrm{N}, 14.90^{\circ}$ E, Friel et al., August 2002; CUMV 88063, tag no. 5197, 64.9 mm SL, sex undetermined, Lékénie River at Mboko landing, Odzala-

Kokua National Park, $0.62^{\circ}$ N, $14.90^{\circ}$ E, Friel et al., August 2002; CUMV 88065, tag no. $5002,70.7 \mathrm{~mm}$ SL, sex undetermined [cytochrome $b$ gene determined], Lékénie River at Mboko landing, Odzala-Kokua National Park, $0.62^{\circ} \mathrm{N}, 14.90^{\circ} \mathrm{E}$, Friel et al., August 2002; CUMV 88064, tag no. 5001, 63.9 mm SL, male [cytochrome $b$ gene determined], Lékénie River at Mboko landing, Odzala-Kokua National Park, $0.62^{\circ} \mathrm{N}$, $14.90^{\circ}$ E, Friel et al., August 2002; CUMV 88052, tag no. 5158, 75.1 mm SL, male, Lékénie River at Mboko landing, Odzala-Kokua National Park, $0.62^{\circ} \mathrm{N}, 14.90^{\circ} \mathrm{E}$, Friel et al., August 2002; CUMV 88053, tag no. 5159, 69.3 mm SL, male, Lékénie River at Mboko landing, Odzala-Kokua National Park, $0.62^{\circ} \mathrm{N}, 14.90^{\circ}$ E, Friel et al., August 2002; CUMV 88123, tag no. 5377, 68.9 mm SL, male, Lékoli River, OdzalaKokua National Park, $0.61^{\circ}$ N, $14.93^{\circ}$ E, Friel et al., August 2002; CUMV 87838, tag no. $5404,90.1 \mathrm{~mm}$ SL, male, Lokoué River, Odzala-Kokua National Park, $0.90^{\circ} \mathrm{N}$, $15.12^{\circ}$ E, Friel et al., August 2002; CUMV 87830, tag no. 5395, 72.3 mm SL, sex undetermined, Lokoué River, Odzala-Kokua National Park, $0.90^{\circ} \mathrm{N}, 15.12^{\circ} \mathrm{E}$, Friel et al., August 2002; CUMV 92390, two specimens, tag no. 6133, 62.8 mm SL, male [cytochrome $b$ gene determined] and tag no. 6134, 67.5 mm SL, male [cytochrome $b$ gene determined], mouth of the Lékéni River near the Lékoli River, Odzala-Kokua National Park, $0.62^{\circ} \mathrm{N}, 14.91^{\circ} \mathrm{E}$, Arnegard et al., June 2006.

Other specimens (18). Republic of the Congo: Cuvette-Ouest: Congo River basin: Lékénie River at Mboko landing, Odzala-Kokua National Park, $0.62^{\circ} \mathrm{N}, 14.90^{\circ} \mathrm{E}$, Friel et al., August 2002: CUMV 88066, tag no. 5028, 59.0 mm SL, sex undetermined; CUMV 88046, tag no. 5126, SL not measured, sex undetermined; Republic of the Congo: Cuvette-Ouest: Congo River basin: Pandaka River, Odzala-Kokua National Park, $0.62^{\circ} \mathrm{N}, 14.92^{\circ}$ E, Friel et al., August 2002: CUMV 88075, tag no. 5075, 71.0 mm SL, sex undetermined; CUMV 88081, tag no. $5084,73.0 \mathrm{~mm}$ SL, male; CUMV 88082, tag no. 5085, 54.0 mm SL , sex undetermined; CUMV 88028, tag no. 5096, 74.0 mm SL, male; CUMV 88029, tag no. 5098, 74.0 mm SL, male; CUMV 88043, tag no. 5122, 73.0 mm SL, male; CUMV 88044, tag no. 5123, 66.0 mm SL, male; CUMV 88045, tag no. 5124, 73.0 mm SL, male; Republic of the Congo: Cuvette-Ouest: Congo River basin: Lokoué River, Odzala-Kokua National Park, $0.90^{\circ} \mathrm{N}, 15.12^{\circ}$ E, Friel et al., August 2002: CUMV 88125, tag no. $5396,74.0 \mathrm{~mm}$ SL, male; Republic of the Congo: Cuvette-Ouest: Congo River basin: small channel around island in Lékoli River, Odzala-Kokua National Park, $0.62^{\circ} \mathrm{N}, 14.92^{\circ} \mathrm{E}$, Friel et al., August 2002: CUMV 88107, tag no. 5276, SL not measured, male; Republic of the Congo: Cuvette-Ouest: Congo River basin: Lékoli River, Odzala-Kokua National Park, $0.61^{\circ} \mathrm{N}, 14.93^{\circ} \mathrm{E}$, Friel et al., August 2002: CUMV 88067, tag no. $5029,59.0 \mathrm{~mm}$ SL, sex undetermined; CUMV 88068, tag no. 5030, 59.0 mm SL, sex undetermined; CUMV 88069, tag no. 5031, 57.0 mm SL, sex undetermined; Republic of the Congo: Cuvette-Ouest: Congo River basin: small stream entering Mambili River from the east between Moba and Lokoué, $0.87^{\circ} \mathrm{N}, 15.11^{\circ} \mathrm{E}$, Friel et al. August 2002: CUMV 88128, tag no. 5423, 70.0 mm SL, male; Democratic Republic of Congo: Orientale Province: Congo basin: BMNH 2013.8.29.34, tag no. JPS-497, 76.0 mm SL , male [cytochrome $b$ gene determined, no EOD recorded], Lifundu River, 5 km downstream of Yangambi,


Figure 5. Photographs of type specimens of Petrocephalus arnegardi sp. n. from Odzala-Kokua National Park, Congo River, Republic of the Congo. Top photograph, a paratype specimen (CUMV 92390, 72.0 mm standard length) before preservation showing appearance in life; middle and bottom photographs represent the left and right sides of the preserved holotype (CUMV 88074, 71.6 mm standard length). Scale bar equals one centimeter.
$0.76^{\circ} \mathrm{N}, 24.24^{\circ} \mathrm{E}$, Lavoué \& Thumitho, 11 September 2010; BMNH 2013.8.29.125, tag no. JPS-511, 48.9 mm SL , sex undetermined [cytochrome $b$ gene determined], Congo River at Yangambi, $0.76^{\circ} \mathrm{N}, 24.24^{\circ} \mathrm{E}$, Lavoué et al., 11 September 2010.

Diagnosis. Petrocephalus arnegardi sp. n. is distinguished from all other Petrocephalus species of Central Africa by the following combination of characteristics. Pigmentation pattern comprising three well-defined, bilateral black patches: one usually distinct (sometimes reduced in size, but rarely absent) round/ovoid subdorsal black mark situated slightly anterior to dorsal, one black mark at the base of each pectoral fin, and one ovoid black mark centered at the base of caudal fin. Dorsal fin at least one third shorter than anal fin (AFL/DFL $\geq 1.5$, range $=1.5-1.7$ ). Dorsal fin with at least 20 branched rays but no more than 22. Anal fin with at least 30 branched rays (range $=30-34$ ). Sixteen teeth or fewer (range $=8-16$ ) in upper jaw, 25 teeth or fewer (range $=20-25$ ) in lower jaw. Eye relatively large (HL/ED $\leq 4.7$, range $=3.5-4.7$ ). Mouth subterminal; ratio of head length to mouth position (HL/MP) between 4.2 and 5.6. Mouth small (HL/MW $\geq 4.1$, range $=4.1-5.0$ ). EOD of normal polarity, mainly biphasic with sometimes the presence of a small-amplitude positive third phase.

Description. This description is based on the material from Odzala-Kokua National Park in the Republic of the Congo. Morphometric ratios and meristic data for the holotype and 17 paratypes are presented in Table 1. Maximum SL observed $=90.1$ mm , holotype $=72.6 \mathrm{~mm})$. Body ovoid, longer than high $(2.3 \leq \mathrm{SL} / \mathrm{H} \leq 2.8$, average $=$ 2.6, holotype $=2.5)$ and laterally compressed. Head length between 3.4 and 4.0 times in standard length (average $=3.6$, holotype $=3.4$ ). Snout short $(6.5 \leq \mathrm{HL} / \mathrm{SNL} \leq 9.3$, average $=7.5$, holotype $=8.3)$ and round. Eye large $(3.5 \leq \mathrm{HL} / \mathrm{ED} \leq 4.4$, average $=4.0$, holotype $=4.1)$. Mouth small $(4.1 \leq \mathrm{HL} / \mathrm{MW} \leq 5.0$, average $=4.6$, holotype $=4.5)$, subterminal, opening under the anterior half of the eye. Teeth small and bicuspid, 8 to 16 (median $=10$ ) in a single row in the upper jaw, 20 to 24 (median $=21$ ) in the lower jaw. Dorsal and anal fins originate in the posterior half of the body $(1.4 \leq S L / P D D \leq 1.6$ and $1.6 \leq \mathrm{SL} / \mathrm{PAD} \leq 1.7$, respectively). Pre-dorsal distance slightly greater than the preanal distance ( $\mathrm{PDD} / \mathrm{PAD}=1.1$ ). Dorsal fin with $20-22$ branched rays (median $=21$ ). Anal fin with 31-34 branched rays (median = 32, holotype $=32$ ). Scales cover the body, except for the head. Lateral line visible and complete with 34 to 38 pored scales along its length. Ten to 13 scales (median $=11$ ), between the anterior base of the anal fin and the lateral line. Caudal peduncle thin $(1.8 \leq \mathrm{CPL} / \mathrm{CPD} \leq 2.5$, average $=2.2$, holotype $=$ 2.2). Twelve scales around the caudal peduncle. Skin on head thick. The three rosettes of Knollenorgans, Augenrosette, Nackenrosette and Kehlrosette, are present on the head.

Electric organ discharge. Statistics for waveform landmarks and other EOD measurements are provided by Lavoué et al. (2008) for specimens recorded in OdzalaKokua National Park, including the holotype and paratypes (specimens listed in Suppl. material 1). Petrocephalus arnegardi sp. n. produces EOD waveforms largely similar to those of many species of this genus. In Odzala-Kokua, mean EOD duration ( $\pm$ std. dev.) is $0.330 \pm 0.074 \mathrm{msec}$ in sexually mature males and $0.270 \pm 0.033 \mathrm{msec}$ in other sex undetermined specimens. The EOD waveform characteristics of the only recorded specimen of $P$. arnegardi sp. n. of Yangambi (Fig. 2B; EOD biphasic, relative height of peak $1=0.216$, relative height of peak $2=-0.784$, duration of peak $1=0.185 \mathrm{msec}$, duration of peak $2=0.075 \mathrm{msec}$, total EOD duration $=0.260 \mathrm{msec}$ ) are similar to those of Odzala-Kokua specimens in all respects. Based on characteristics of the EODs,
the electrocytes are assumed to have non-penetrating stalks and to be innervated posteriorly (Sullivan et al. 2000).

Live coloration (Fig. 5; see also Fig. 3 in Lavoué et al. 2010). Body uniformly silvery white, with three distinct bilateral melanin marks: a distinct, ovoid black mark situated slightly anterior to the dorsal fin, sometimes covering only a few scales, a black spot at the base of the pectoral fin and a somewhat vertically oriented ovoid black mark centered at the base of the caudal fin that does not extend onto the upper and lower parts of the caudal fin. Fins hyaline.

Preserved coloration (Fig. 5; see also Fig. 3 in Lavoué et al. 2010). Body light brown, with head and dorsum slightly darker. Thick skin on head opaque. Numerous light melanophores on body, slightly larger ventrally from the snout to the anal fin. Fins whitish/opaque.

Distribution (Fig. 1). Endemic to the Congo River basin. Holotype and paratypes from Odzala-Kokua National Park (Republic of the Congo) where they were mainly collected along the main channel of the Lékoli River, northwestern Congo River basin. The two specimens collected at Yangambi will extend the distribution to the eastern part of the Congo basin's central cuvette. Abundant in Odzala-Kokua but apparently rare at Yangambi (Poll and Gosse 1963).

Phylogenetic affinities (Fig. 4). The Yangambi specimens and the Odzala-Kokua specimens of $P$. arnegardi sp. n. are sister groups in the cytochrome $b$ phylogenetic tree. Petrocephalus arnegardi sp. n. belongs to a clade containing $P$. odzalaensis and $P$. balayi, two other species with a similar melanin pattern composed of three distinct black marks, but it is only distantly related to $P$. boboto sp. n. As previously noted, $P$. binotatus is absent in this tree and its phylogenetic position is unknown.

Etymology. This species is dedicated to Matthew E. Arnegard, our friend and colleague, in recognition of his contributions to study of mormyrid evolution and diversification (e.g., Arnegard et al. 2005; Arnegard and Carlson 2005; Arnegard et al. 2010a; Arnegard et al. 2010b). Matthew Arnegard is additionally a member of the "Mintotom Team": researchers associated with the Carl D. Hopkins Laboratory at Cornell University who have conducted field studies on African weakly electric fishes for more than 15 years. ("Mintotom" is the plural form of the word for mormyrid fish in the Fang language of West Central Africa.).

Comparisons. As for $P$. boboto sp. n., the presence of three dark spots in $P$. arnegardi sp. n. distinguishes this species from most of its congeners. As for other species having a similar pattern of melanin marking, $P$. arnegardi sp. n. can easily be distinguished from P. zakoni by the presence of three electroreceptor rosettes on the head (versus absence of all three in $P$. zakoni) and a higher number of anal fin rays (a minimum of 30 in $P$. arnegardi sp. n. versus a maximum of 28 in $P$. zakoni). Its high number of anal fin rays distinguishes $P$. arnegardi sp. n. from $P$. balayi and $P$. odzalaensis (30-34 in P. arnegardi sp. n. versus a maximum of 27 and 20 in P. balayi and P. odzalaensis, respectively). Petrocephalus balayi has a proportionally larger mouth (HL/MW $=2.7-3.9$ versus $4.1-5.0$ in $P$. arnegardi sp. n.). Petrocephalus arnegardi sp. n. generally resembles to the holotype of $P$. binotatus, leading Lavoué et al. (2010) to assign these specimens from the Odzala-


Figure 6. Top photograph, the preserved holotype of Petrocephalus binotatus (MRAC 15191; 83.2 mm standard length) collected at Ikengo near the modern locality of Mbandaka, Equateur Province, in the Central Congo basin of Democratic Republic of Congo (Photo by Wilhelm Harder, reproduced courtesy of ETI Bioinformatics); bottom photograph, a specimen of Petrocephalus arnegardi (BMNH 2013.8.29.34) before preservation showing appearance in life, from Yangambi, Congo River, Democratic Republic of Congo.

Kokua National Park to P. binotatus. However, Petrocephalus arnegardi sp. n. has a distinctly smaller mouth than $P$. binotatus (HL/MW=4.4-5.2 in Odzala-Kokua specimens and 4.2 and 4.5 in the two Yangambi specimens versus 3.2 in the holotype of $P$. binota$t u s$ ) and a smaller interorbital width (HL/IOW $\geq 2.6$ in Odzala-Kokua and Yangambi specimens versus 2.3 in the holotype of $P$. binotatus). The faded pigmentation in the pre-
served holotype of $P$. binotatus precludes its accurate description and comparison (Fig. 6). Whereas a faded roundish black mark situated slightly anterior to the dorsal fin on each side of the flank and an ovoid black mark centered at the base of the caudal fin are visible on the preserved holotype, the presence of a black mark at the base of the pectoral fin is ambiguous (Fig. 6). In his description of $P$. binotatus, Pellegrin (1924) mentioned the subdorsal mark and the mark at the base of the anal fin, but did not make reference to any black mark at the origin of the pectoral fin. The black mark at the base of the pectoral fin in $P$. arnegardi sp. n. is always present and intense. Pellegrin also described the black mark at the base of the caudal fin as crescent-like, extending onto the upper and lower rays of this fin (see drawing in Pellegrin 1928) whereas on the holotype this mark appears more ovoid and does not seem to extend onto any fin rays (Fig. 6). Petrocephalus arnegardi sp. n . is distinguished from $P$. boboto sp. n. by a distinctly smaller mouth (SL/ MW $\geq 14.7$, range $=14.7-18.4$ versus 14.2 in $P$. boboto sp. n.), a slightly larger interorbital distance ( $\mathrm{HL} / \mathrm{IOW} \leq 3.9$, mean $=3.2$ versus 3.7 in $P$. boboto sp. n.) and the presence of a well-defined Augenrosette (versus reduced in $P$. boboto sp. n.). In our phylogenetic tree (Fig. 4), P. boboto sp. n. is not the sister group of $P$. arnegardi sp. n.

## Key to the Petrocephalus species of the Central Congo basin

1 Dorsal fin with fewer than 18 branched rays (rarely 18); only eight to 10 scale rows between the anterior base of the anal fin and the lateral line; distinct melanin markings absent on body (i.e., absence of black patches that are spe-cies-specific for many other Petrocephalus species); rosettes of Knollenorgan electroreceptors absent on the head....Petrocephalus microphthalmus group Note: Currently, the Petrocephalus microphthalmus group contains three morphologically similar valid species: P. microphthalmus, Petrocephalus haullevillii, and Petrocephalus schoutedeni. The identification of each of these three species is currently challenging pending their taxonomic revision.

- Dorsal fin usually with more than 20 branched rays (sometimes 20; in very rare instances 19); at least 10, usually more, scale rows between the anterior base of the anal fin and the lateral line; distinct melanin markings (black patches) may be present or absent on the body; electroreceptor rosettes present or absent on the head2

2 Mouth large, its width at most 3.9 times in head length; at least 15 teeth in upper jaw, usually more3

- Mouth small, its width at least 3.6 times (usually $4.0-4.4$ times) in head length; usually fewer than 15 teeth in upper jaw (rarely 15 or 16)............... 5
3 Anal fin contains 26 or 27 branched rays; 20-22 branched rays in the dorsal fin; three bilateral intense black patches present: a rounded sub-dorsal mark, an ovoid caudal mark and a mark at the origin of the pectoral fin

Petrocephalus balayi

- Anal fin with more than 30 branched rays; more than 24 branched rays in dorsal fin; only two distinct, bilateral black patches: a sub-dorsal mark and a caudal mark
Eye relatively small ( $\mathrm{HL} / \mathrm{ED} \geq 4.0$ ); mouth subterminal (HL/MP $\geq 4.4$ ), opening under the anterior half of the eye; two distinct melanin marks present but sometimes pale: a rounded sub-dorsal mark and a crescent-like mark at the base of the caudal fin; Knollenorgan electroreceptors organized into three distinct rosettes on head, but rosettes relatively small; EOD waveform typical for the genus, polarity normal (see Fig. 10C in Lavoué et al. 2010) ...


## Petrocephalus sauvagii

- Eye large ( $\mathrm{HL} / \mathrm{ED} \leq 3.5$ ); mouth subterminal but positioned more posteriorly along the ventral margin of the head ( $\mathrm{HL} / \mathrm{MP} \leq 3.5$ ), opening under the posterior half of the eye; two distinct melanin marks: a rounded, sometimes irregularly shaped, sub-dorsal black mark and a crescent-like black mark at the base of the caudal fin; three larger rosettes of Knollenorgan electroreceptors present on the head; EOD waveform very distinctive among congeners, appearing to be reversed in polarity compared to EODs of all other Petrocephalus species (see Fig. 11C in Lavoué et al. 2010) .........Petrocephalus pulsivertens Anal fin contains 30 or more branched rays; melanin markings (black patches) present on the body and always distinctly visible.6
- Anal fin contains at most 29 branched rays (usually fewer); melanin markings present but sometimes hardly visible. 9
6 Dorsal fin contains 24-26 branched rays; eye large (HL/ED $\leq 3.2$ ); mouth very small relative to many congeners (HL/MW $\geq 5.2$ ); two melanin marks present and distinct but of medium intensity: a rounded sub-dorsal mark and a crescent-like mark at the base of the caudal fin; two readily observable rosettes of Knollenorgan electroreceptors present on the head (Augenrosette and Nackenrosette) plus a Kehlrosette that is rather difficult to observe without staining.

Petrocephalus grandoculis

- Dorsal fin contains 24 or fewer branched rays; eye smaller in size ( $3.5 \leq \mathrm{HL} /$ $\mathrm{ED} \leq 4.7$ ); mouth larger (HL/MW $\leq 5.2$ ); three distinct melanin marks (black patches) present: an ovoid sub-dorsal mark (sometimes small but easily discernable), an ovoid mark at the base of the caudal fin and a mark at the origin of the pectoral fin; Nackenrosette and Kehlrosette present on the head and distinct; Augenrosette present but sometimes reduced in size to few, not densely packed, Knollenorgan pores
$7 \quad$ Augenrosette present but reduced in size to few Knollenorgan pores; narrow head $(\mathrm{HL} / \mathrm{HW}=2.4$ and $\mathrm{HL} / \mathrm{IOW}=3.7)$.......Petrocephalus boboto sp. $\mathbf{n}$.
- $\quad$ Augenrosette present and well distinct; wider head (HL/HW $\leq 2.2$ and HL/ IOW $\leq 3.9$, average $=3.2$ )$(\mathrm{HL} / \mathrm{IOW}=2.3) ; 24$ dorsal fin branched rays....... $P$

Petrocephalus binotatus

Smaller mouth (SL/MW $\geq 14.7$ and HL/MW $\geq 4.1$ ); shorter interorbital distance (HL/IOW $\geq 2.6$ ); 22 or fewer dorsal fin branched rays

## Petrocephalus arnegardi sp. n.


$\square$
Mouth subterminal, opening under the anterior half of the eye; snout short (HL/SNL $\geq 6.5$ ); Knollenorgan electroreceptors on head are clustered into three rosettes (but a distinctive Kehlrosette is difficult to observe without staining); EOD of normal polarity, often appearing to have an overall biphasic waveform at low gain, although a minute third peak is in fact present (first head-positive peak, P1, much larger in amplitude than second head-positive peak, P3, which never exceeds $10 \%$ of total peak-to-peak amplitude)....Petrocephalus valentini Mouth subterminal but positioned more caudally along ventral margin of head, opening under posterior half of eye; snout somewhat longer (HL/SNL $=5.4$ in single specimen available, holotype); Knollenorgans on head clustered into only two rosettes (Nackenrosette and the Kehlrosette), Augenrosette absent; EOD of normal polarity, with more than two phases apparent even at low gain (the only specimen recorded exhibits an EOD containing 4 peaks; the second head-positive peak, P 3 , is larger in amplitude than the first head-positive peak, P1; amplitude of P3 substantially greater than $10 \%$ of total peak-to-peak amplitude).
.Petrocephalus mbossou

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## Supplementary material I

## List of specimens of Petrocephalus arnegardi and Petrocephalus boboto with EOD recording

Authors: Sébastien Lavoué, John P. Sullivan
Data type: species data
Explanation note: List of specimens of Petrocephalus arnegardi and Petrocephalus boboto with electric organ discharge (EOD) recording (holotypes, paratypes, non types).
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Link: doi: 10.3897/zookeys.400.6743.app1

# A new species of the paper wasp genus Polistes (Hymenoptera, Vespidae, Polistinae) in Europe revealed by morphometrics and molecular analyses 

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[^0]http://zoobank.org/91DC4784-F49A-4353-B4C8-DC0F67B1EF92
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#### Abstract

We combine multivariate ratio analysis (MRA) of body measurements and analyses of mitochondrial and nuclear data to examine the status of several species of European paper wasps (Polistes Latreille, 1802) closely related to P. gallicus. Our analyses unambiguously reveal the presence of a cryptic species in Europe, as two distinct species can be recognized in what has hitherto been considered Polistes bischoffi Weyrauch, 1937. One species is almost as light coloured as P. gallicus, and is mainly recorded from Southern Europe and Western Asia. The other species is darker and has a more northern distribution in Central Europe. Both species occur syntopically in Switzerland. Given that the lost lectotype of $P$. bischoff originated from Sardinia, we selected a female of the southern species as a neotype. The northern species is described as P. helveticus sp. n. here. We also provide a redescription of $P$. bischoffi rev. stat. and an identification key including three more closely related species, P. biglumis, P. gallicus and P. hellenicus.


## Keywords

Cytochrome oxidase 1, ITS, DNA barcoding, principal component analysis, shape PCA, linear discriminant analysis, LDA ratio extractor, ratio spectrum, allometry, cryptic species, Switzerland

## Introduction

The paper wasp genus Polistes Latreille, 1802 (Hymenoptera, Vespidae, Polistinae) is an important model group for behavioral and evolutionary studies. It includes a large number of eusocial species that exhibit varied forms of social organization (West-Eberhard 1969). Moreover, its comparatively small colony size and exposed nests facilitate both field observations and experiments (e.g., Cervo et al. 2008). More than 220 species are currently recognized worldwide (Arens 2011, Buck et al. 2012, Nugroho et al. 2012: 72), ten of which occur in Europe (Arens 2011: 462, Carpenter 1997: 142, Castro and Dvořák 2009: 300). Three of them, namely P. atrimandibularis Zimmermann, 1930, P. semenowi Morawitz, 1889, and P. sulcifer Zimmermann, 1930, are social parasites (Cervo 2006, and references therein) and were considered as members of a distinct genus (or subgenus) Sulcopolistes Blüthgen, 1938 (Blüthgen 1961, Guiglia 1972), until Carpenter (1990) synonymized Sulcopolistes with Polistes. Later, phylogenetic analyses of one mitochondrial gene fragment showed that the three socially parasitic species formed a monophyletic group nested within other European Polistes (Choudhary et al. 1994: 33); the three social parasites constituted a monophyletic clade sister to a clade consisting of $P$. dominula (Christ, 1791) and $P$. nimpha (Christ, 1791).

Blüthgen (1943) proposed the subgeneric name Leptopolistes for several non-parasitic European species, including P. associus (Kohl, 1898), the type species of Leptopolistes, as well as P. bischoffi Weyrauch, 1937 and P. gallicus (Linnaeus, 1767). Males of these taxa share non-convex, immediately narrowing genae, as seen in dorsal view (Blüthgen 1943: 99; Guiglia 1972: 49), giving the male head a characteristically slender aspect. Currently, all European Polistes species are assigned to the subgenus Polistes (Carpenter 1996b), although the species formerly included in Leptopolistes species are still considered to be closely related (Carpenter 1997).

In fen rotational fallows (Gigon et al. 2010) at the shore of Lake Greifen [Greifensee] in the Swiss midlands Neumeyer et al. (2011) found a population of paper wasps that could not be assigned to any described species. This taxon is colored almost as light as Polistes gallicus (Linnaeus, 1767) and was therefore tentatively called "Polistes cf. gallicus" by Neumeyer et al. (2011). Polistes gallicus is quite common in Southern Europe, but it does not usually occur in wetlands and is not known as far north in Switzerland. The unidentified taxon from the Swiss midlands, however, shares an important trait (a reduced epicnemial carina) with another taxon that has hitherto been referred to as Polistes bischoffi Weyrauch, 1937 (e.g. Blüthgen 1961, Guiglia 1972, Mauss and Treiber 2004, Dvořák and Roberts 2006, Witt 2009), a common wetlanddweller in Switzerland and other countries of Central Europe.

To resolve the identity of the unidentified taxon from the Swiss wetlands, we examine its affinity to other European species using a combination of morphological, morphometric and molecular analyses. Recently, Buck et al. (2012) unraveled cryptic diversity in the Nearctic subgenus Fuscopolistes Richards, 1973 using multivariate morphometrics and DNA barcoding. In contrast to their study, we used a nuclear marker in addition to the mitochondrial marker and multivariate ratio analysis (MRA) instead of classic multivariate methods. MRA is a recently developed extension of principal component analysis (PCA) and linear discriminant analysis (LDA) that was specifically designed for the exploration of body measurements in a taxonomic context (Baur and Leuenberger 2011, László et al. 2013).

Our analyses lead to the recognition of two distinct species within what has been hitherto referred to as $P$. bischoff; we review the information on the type material of bischoff, and designate a neotype to settle the status of this species. P. bischoff turns out to be the valid name of the unidentified taxon ("cf. gallicus") found close to Zurich by Neumeyer et al. (2011); a new name is required for the species referred to as bischoffi by some authors (Blüthgen 1961, Guiglia 1972, Mauss and Treiber 2004, Dvorák and Roberts 2006, Witt 2009): P. helveticus, which is described here. Lastly, we provide an identification key that, in combination with available keys (Mauss and Treiber 2004, Dvořák and Roberts 2006, Witt 2009), will facilitate the identification of the Central European species.

## Material and methods

For the molecular and morphometric analyses we focus on the status of the two closely related morphs hitherto comprised under Polistes bischoff (see introduction), as well as on the morphologically similar Polistes gallicus, and on their separation from other European Polistes. At this stage of the analyses, we deliberately avoid the concept of species and rather interprete them in the sense of operational taxonomic units, hereafter called "OTUs". The OTUs are labeled with their valid taxonomic names (Carpenter 1996b), except for the two taxa hitherto comprised under Polistes bischoff which are labeled in a manner that already anticipates the outcome of our study and our neotype designation. Detailed information on the taxonomic status of these names will be provided after the presentation of the results from the molecular and morphometric analyses.

## Molecular analyses

## a) Species included

Ninety-nine specimens were included in the molecular analysis, representing eleven OTUs. In addition, two specimens each of Vespula germanica (Fabricius, 1793) and $V$. vulgaris (Linnaeus, 1758) were used to root the trees; sequences for Polistes (Polistella) snelleni Saussure, 1862 and Polistes (Aphanilopterus) exclamans Viereck, 1906 were
downloaded from Genbank and used with the two species of Vespula to root the trees in analyses of the mitochondrial sequences. Most specimens were collected in $80 \%$ ethanol in the field, but we also included some specimens that were killed with ethyl acetate. For specimens collected before 2012, DNA was extracted from the mesosoma, leaving the legs, wings, head and metasoma as vouchers; for specimens collected in 2012 and 2013, as well as specimens selected as type specimens, DNA was extracted from one single leg to preserve a nearly intact specimen. Most specimens were collected in Switzerland, but we also included specimens form Croatia, France, Greece, Italy and Portugal (Table 1). All DNA extractions are deposited in the DNA bank of the Swiss Barcode of Life initiative (Swissbol; www.swissbol.ch).

## b) Lab protocols

Full lab protocols can be found in Praz et al. (2008). DNA was isolated using phe-nol-chloroform extractions; PCR reactions were performed with GoTaq polymerase (Promega) in a Biometra T1 thermocycler. PCR products were purified enzymatically using a mix of the enzymes exonuclease I (Fermentas) and FastAP thermosensitive alkaline phosphatase (Fermentas) and sequenced in both directions with the primers used in the original amplification using BigDye terminator technology (Applied Biosystems). Big Dye products were purified with Sephadex (GE Healthcare Life Sciences) and analyzed on a ABI-3500 DNA sequencer.

## c) Markers and primers

We sequenced two fast-evolving genetic markers: the 600 bp fragment of the mitochondrial gene cytochrome oxidase 1 (COX1) used as an universal barcode (Hebert et al. 2003) and the nuclear marker ITS1; we chose ITS1 rather than ITS2 because preliminary analyses revealed that ITS2 was polymorphic in $P$. bischoff and could not be sequenced directly.

For COX1 we used the universal primers LepF and LepR (Hebert et al. 2004) with the following conditions: an initial denaturation of 1 min at $94^{\circ} \mathrm{C}$, then six cycles of 1 min at $94^{\circ} \mathrm{C}, 1.5 \mathrm{~min}$ at $45^{\circ} \mathrm{C}$, and 1.25 min at $72^{\circ} \mathrm{C}$, followed by 36 cycles of 1 min at $94^{\circ} \mathrm{C}, 1.5 \mathrm{~min}$ at $51^{\circ} \mathrm{C}$, and 1.25 min at $72^{\circ} \mathrm{C}$, with a final step of 5 min at $72^{\circ} \mathrm{C}$. For specimens with degraded DNA, we used another universal forward primer, UAE3 (Zhang and Hewitt 1996) in combination with LepR to amplify a 400 bp fragment of the barcode. The conditions for this 400 bp fragment were as above, except that the extension time at $72{ }^{\circ} \mathrm{C}$ was 45 seconds in each cycle.

The presence of nuclear pseudogenes, or NUMTs, was carefully examined by visually detecting "ghost bands" on the agarose gel, and especially by detecting double peaks in the chromatograms. No indication of the presence of NUMTs was found in the specimens analyzed, with the exception of $P$. nimpha. For this OTU, double peaks were found in up to 20 nucleotide positions in every specimen, strongly suggesting the presence of NUMTs; no indels were found, and no stop codons were found in the translated amino acid sequence for these sequences, even when polymorphism was allowed, suggesting that the NUMTs were highly similar to the true mitochondrial sequences

Table I. Locality information, voucher numbers and GenBank accession numbers for sequences used in this study.

| Unit name | Voucher No | COX1 | ITS1 | Locality |
| :---: | :---: | :---: | :---: | :---: |
| Outgroup |  |  |  |  |
| Vespula germanica | 39 | KJ415826 | KJ415926 | I, Crevoladossola |
| Vespula germanica | 90 | KJ415827 | KJ415927 | CH , Zürich |
| Vespula vulgaris | 79 | KJ415828 | KJ415928 | CH, Pfäffikon |
| Vespula vulgaris | 89 | KJ415829 | KJ415929 | CH , Zürich |
| Polistes snelleni | - | EF136457 | - | - |
| Polistes exclamans | - | JN988655 | - | USA, Florida, Archbold |
| Ingroup |  |  |  |  |
| Polistes associus | 7 | KJ415830 | KJ415930 | HR, Rovinj |
| Polistes associus | 286 | KJ415831 | KJ415931 | CH, Losone |
| Polistes biglumis | 21 | KJ415832 | KJ415932 | CH, Val Müstair, Tschierv |
| Polistes biglumis | 27 | KJ415833 | KJ415933 | CH, Val Müstair, Sta. Maria |
| Polistes biglumis | 28 | KJ415834 | KJ415934 | CH, Val Müstair, Müstair |
| Polistes biglumis | 29 | KJ415835 | KJ415935 | CH, Val Müstair, Tschierv |
| Polistes biglumis | 87 | KJ415836 | KJ415936 | CH, Val Müstair, Tschierv |
| Polistes bischoffi | 1 | KJ415837 | KJ415937 | CH, Pfäffikon |
| Polistes bischoff | 22 | KJ415838 | KJ415938 | CH, Pfäffikon |
| Polistes bischoff | 75 | KJ415839 | KJ415939 | CH, Wetzikon |
| Polistes bischoff | 76 | KJ415840 | KJ415940 | CH, Wetzikon |
| Polistes bischoff | 77 | KJ415841 | - | CH, Wetzikon |
| Polistes bischoffi | 82 | KJ415842 | KJ415941 | CH, Wetzikon |
| Polistes bischoff | 83 | - | KJ415942 | CH, Wetzikon |
| Polistes bischoff | 105 | KJ415843 | KJ415943 | CH, Wetzikon |
| Polistes bischoff | 135 | KJ415844 | KJ415944 | CH, Greifensee |
| Polistes bischoff | 136 | KJ415845 | KJ415945 | CH, Greifensee |
| Polistes bischoff | 137 | KJ415846 | KJ415946 | CH, Mönchaltorf |
| Polistes bischoff | 366 | KJ415847 | KJ415947 | F, Corsica, Galeria |
| Polistes dominula | 2 | KJ415848 | KJ415948 | CH, Weiach |
| Polistes dominula | 4 | KJ415849 | KJ415949 | CH, Weiach |
| Polistes dominula | 5 | KJ415850 | KJ415950 | CH, Weiach |
| Polistes dominula | 6 | KJ415851 | KJ415951 | CH, Stallikon |
| Polistes dominula | 15 | KJ415852 | KJ415952 | CH, Wetzikon |
| Polistes dominula | 16 | KJ415853 | KJ415953 | CH, Wetzikon |
| Polistes dominula | 25 | KJ415854 | KJ415954 | CH, Val Müstair, Sta. Maria |
| Polistes dominula | 26 | KJ415855 | KJ415955 | CH, Val Müstair, Sta. Maria |
| Polistes dominula | 30 | KJ415856 | KJ415956 | CH, Grono |
| Polistes dominula | 31 | KJ415857 | KJ415957 | CH, Grono |
| Polistes dominula | 32 | KJ415858 | KJ415958 | CH, Grono |
| Polistes dominula | 40 | KJ415859 | KJ415959 | I, Masera |
| Polistes dominula | 43 | KJ415860 | KJ415960 | CH, Leuk |
| Polistes dominula | 44 | KJ415861 | KJ415961 | CH, Leuk |
| Polistes dominula | 52 | KJ415862 | KJ415962 | CH, Noville |
| Polistes dominula | 54 | KJ415863 | KJ415963 | CH , Meinier |
| Polistes dominula | 58 | KJ415864 | - | CH, Cudrefin |
| Polistes dominula | 63 | KJ415865 | KJ415965 | CH, Cudrefin |


| Unit name | Voucher No | COX1 | ITS1 | Locality |
| :---: | :---: | :---: | :---: | :---: |
| Polistes gallicus | 9 | KJ415866 | KJ415966 | HR, Rovinj |
| Polistes gallicus | 41 | KJ415867 | KJ415967 | CH, Leuk |
| Polistes gallicus | 42 | KJ415868 | KJ415968 | CH, Leuk |
| Polistes gallicus | 103 | KJ415869 | KJ415969 | CH, Sant Antonino |
| Polistes gallicus | 108 | KJ415870 | KJ415970 | P, Vila do Bispo |
| Polistes gallicus | 115 | KJ415871 | KJ415971 | CH, Villars-sous-Yens |
| Polistes gallicus | 118 | KJ415872 | KJ415972 | CH, San Vittore |
| Polistes gallicus | 343 | KJ415873 | KJ415973 | I, Cabras |
| Polistes gallicus | 344 | KJ415874 | KJ415974 | I, Macomer |
| Polistes gallicus | 345 | KJ415875 | KJ415975 | I, Scano di Montiferro |
| Polistes gallicus | 346 | KJ415876 | KJ415976 | I, Tadasuni |
| Polistes gallicus | 347 | KJ415877 | KJ415977 | I, Cabras |
| Polistes gallicus | 348 | KJ415878 | KJ415978 | I, Scano di Montiferro |
| Polistes sp. aff. gallicus | 126 | KJ415879* | - | GR, Ano Kotili |
| Polistes sp. aff. gallicus | 129 | KJ415880* | - | GR, Olympia |
| Polistes hellenicus | 8 | KJ415881 | KJ415979 | HR, Rovinj |
| Polistes hellenicus | 10 | KJ415882 | KJ415980 | HR, Rovinj |
| Polistes hellenicus | 11 | KJ415883 | KJ415981 | HR, Rovinj |
| Polistes hellenicus | 88 | - | KJ415982 | HR, Rovinj |
| Polistes hellenicus | 96 | KJ415884 | KJ415983 | GR, Zacharo |
| Polistes hellenicus | 101 | KJ415885 | KJ415984 | HR, Vela Učka |
| Polistes hellenicus | 102 | KJ415886 | KJ415985 | HR, Vela Učka |
| Polistes hellenicus | 133 | KJ415887 | - | GR, Avia bei Kalamata |
| Polistes helveticus | 3 | KJ415888 | KJ415986 | CH, Pfäffikon |
| Polistes helveticus | 12 | KJ415889 | KJ415987 | CH, Wetzikon |
| Polistes helveticus | 13 | KJ415890 | KJ415988 | CH, Wetzikon |
| Polistes helveticus | 14 | KJ415891 | KJ415989 | CH, Wetzikon |
| Polistes helveticus | 17 | KJ415892 | KJ415990 | CH, Wetzikon |
| Polistes helveticus | 18 | KJ415893 | KJ415991 | CH, Pfäffikon |
| Polistes helveticus | 19 | KJ415894 | KJ415992 | CH, Pfäffikon |
| Polistes helveticus | 20 | KJ415895 | KJ415993 | CH, Pfäffikon |
| Polistes helveticus | 33 | - | KJ415994 | CH , Seegräben |
| Polistes helveticus | 34 | KJ415896 | KJ415995 | CH, Seegräben |
| Polistes helveticus | 35 | KJ415897 | KJ415996 | CH, Wetzikon |
| Polistes helveticus | 46 | KJ415898 | KJ415997 | CH , Noville |
| Polistes helveticus | 47 | KJ415899 | KJ415998 | CH, Noville |
| Polistes helveticus | 48 | KJ415900 | KJ415999 | CH, Noville |
| Polistes helveticus | 49 | KJ415901 | KJ416000 | CH, Noville |
| Polistes helveticus | 50 | KJ415902 | KJ416001 | CH, Noville |
| Polistes helveticus | 51 | KJ415903 | KJ416002 | CH, Noville |
| Polistes helveticus | 78 | KJ415904 | KJ416003 | CH, Pfäffikon |
| Polistes helveticus | 81 | KJ415905 | - | CH, Wetzikon |
| Polistes helveticus | 138 | KJ415906 | KJ416004 | CH, Schwerzenbach |
| Polistes helveticus | 139 | KJ415907 | - | CH, Schwerzenbach |
| Polistes nimpha | 53 | KJ415908 | - | CH, Noville |
| Polistes nimpha | 55 | KJ415909 | - | CH, Chabrey |
| Polistes nimpha | 56 | KJ415910 | - | CH, Chabrey |
| Polistes nimpha | 57 | KJ415911 | KJ416005 | CH, Cudrefin |


| Unit name | Voucher No | COX1 | ITS1 | Locality |
| :---: | :---: | :---: | :---: | :---: |
| Polistes nimpha | 59 | KJ415912 | - | CH, Cudrefin |
| Polistes nimpha | 60 | KJ415913 | - | CH, Cudrefin |
| Polistes nimpha | 61 | KJ415914 | - | CH, Cudrefin |
| Polistes nimpha | 65 | KJ415915 | KJ416006 | CH, Chabrey |
| Polistes nimpha | 66 | KJ415916 | - | CH, Chabrey |
| Polistes nimpha | 67 | KJ415917 | - | CH, Cudrefin |
| Polistes nimpha | 68 | KJ415918 | - | CH, Cudrefin |
| Polistes nimpha | 69 | KJ415919 | - | CH, Cudrefin |
| Polistes nimpha | 85 | KJ415920 | - | CH, Meride |
| Polistes nimpha | 86 | KJ415921 | - | I, Valsolda |
| Polistes semenowi | 296 | KJ415922 | KJ416007 | CH, Gampel |
| Polistes sulcifer | 119 | KJ415923 | KJ416008 | HR, Vela Učka |
| Polistes sulcifer | 120 | KJ415924 | KJ416009 | HR, Vela Učka |
| Polistes sulcifer | 134 | KJ415925 | KJ416010 | CH, Semione |

* sequenced with UAE3/LepR instead of LepF/LepR
and thus of recent origin. The presence of NUMTs in $P$. nimpha was therefore unlikely to affect our results, especially given that $P$. nimpha was not the focus of our study, as it is not closely related to any of the main OTUs.

For ITS1, we used the primers CAS18sF1 and CAS5p8sB1d (Ji et al. 2003) to amplify a 700 bp fragment. For most specimens, the chromatograms were clean, without double peaks, indicating no within-specimen polymorphism in ITS1. In P. dominula, a few sites were polymorphic, and one insertion rendered the sequencing difficult in some specimens at position 550; in $P$. nimpha, several sites were polymorphic and insertions or deletions prevented direct sequencing in all specimens, except two (the numbers 57 and 65). Given that $P$. nimpha was not the focus of our study, we did not clone the PCR products to obtain clean sequences of the individual copies of ITS1, and merely included two specimens in our analysis.

## d) Analyses

Genetic distances between each terminal were computed under the GTR model of nucleotide substitution in Paup 4.0b10 (Swofford 2002). We then performed maximum likelihood analyses of each marker separately using RAXML (Stamatakis et al. 2005), performing 1000 bootstrap replicates. For the mitochondrial marker, the first and second position were combined in one partition, while the third codon position constituted a second partition. For ITS1, we coded each insertion or deletion as an additional, binary character added as a separate partition, hereafter referred to as the "gap" partition; one insertion or deletion was considered as one character, regardless of the size of the indel. In total, the coding of the insertions and deletions resulted in 42 characters, 38 of which were parsimony informative and four of which were autapomorphic. We do not intend to unravel the phylogenetic relationships among the European species of Polistes, and therefore we do not present an analysis of a matrix combining both genes.

We applied a GTR + G model to each DNA partition; the gap partition was analyzed as a binary character with two states, with a gamma shape to accommodate rate heterogeneity. FigTree v1.3.1 (Rambaut 2009) was used to visualize the trees and produce the figures.

## Morphometrics

We restricted the morphometric analyses to the five most morphologically similar OTUs, namely biglumis, bischoffi, gallicus, hellenicus, and helveticus. For convenience, we refer hereafter to this group as the gallicus-group. We stress that we consider this group to be neither monophyletic nor taxonomically relevant.

## a) Character selection and measurements

We measured a total of 266 specimens, most of them from Switzerland (158), but also some from Italy (30), Greece (24), Croatia (17), France (10), Germany (6), Slovakia (3), Turkey (3), Czech Republic (2), Liechtenstein (2), Austria (1), Azerbaijan (2), Uzbekistan or Tadjikistan (3), Mongolia (2), China (1), and Portugal (1). Sixteen characters were selected (Table 2) for measurements, most of them on the head and antenna, and two on the hind leg. Measurements were made on mounted specimens or parts (head, leg) of them using a pinholding device, permitting rotations around all three axes (X, Y, and Z). An Olympus SZH10 stereo-microscope equipped with eye-pieces Olympus GWH10X-D (with an eye-piece micrometer dividing 10 mm in 100 units) and Leica 10445111 (10x/21B) (with an eye-piece micrometer dividing 5 mm in 100 units) was used at several magnifications (Table 2). For terminology of morphological structures we followed Goulet and Huber (1993), occasionally also Richards (1973).

## b) Morphometric analysis

We applied the multivariate ratio analysis (MRA) of Baur and Leuenberger (2011) to our data. MRA comprises a set of tools for analyzing size and shape of body measurements in a multivariate mathematical framework that is entirely consistent with the customary usage of body lengths and ratios in taxonomic works (e.g., in descriptions, diagnoses). In systematic and taxonomic studies, MRA offers several advantages over conventional explorative multivariate methods, such as principal component analysis (PCA) and linear discriminant analysis (LDA). MRA removes biases from spurious contradictions in the results due to different definitions of size and shape. Furthermore, the numeric output of MRA can be used directly in the descriptive part of a taxonomic study. László et al. (2013) reviewed these issues in an application to parasitic wasps. Following Baur and Leuenberger (2011), we first calculated isometric size (isosize), defined as the geometric mean of all variables. We then performed a shape PCA (i.e., a principal component analysis in the space of all ratios) for evaluating how the morphometric pattern corresponds to the OTUs revealed in the molecular analyses. In order to decide how many components to retain we inspected the scree

Table 2. Definition of distance measurements (* the Leica eye-piece micrometer dividing 5 mm in 100 units was used).

| abbreviation | term | definition of measurement | magnification |
| :---: | :---: | :---: | :---: |
| cly.b | clypeus breadth | minimal distance between inner eye orbits | $70 \times$ |
| eye.d | eye distance | minimal distance between inner eye orbits, dorsal view | $50 \times$ |
| eye.h | eye height | height of eye in antero-lateral view | $30 \times$ |
| flgfirst. 1 | first flagellomere length | length of first flagellomere, outer upper aspect | $70 \times$ |
| flglast.b | terminal flagellomere breadth | breadth of terminal flagellomere, inner lateral aspect | *70x |
| flglast.1 | terminal flagellomere length | length of terminal flagellomere (10th in female, 11th in male), inner lateral aspect | *70× |
| hea.b | head breadth | head breadth, dorsal view | $30 \times$ |
| hea.h | head height | clypeal apex to anterior margin of median ocellus | $30 \times$ |
| lof. 1 | lower face length | clypeal apex to lower margin of toruli | 50x |
| msp. 1 | malar space | distance between lower eye orbit and mouth margin according to Arens (2011) | *70× |
| ool. 1 | lateral ocellus to eye distance | minimal distance between lateral ocellus and upper eye orbit | *70x |
| pol. 1 | lateral ocelli distance | minimal distance between lateral ocelli | *70x |
| scp.b | scape breadth | breadth of scape, dorsal view | *70x |
| scp.l | scape length | length of scape, inner lateral aspect | $70 \times$ |
| tib3.b | metatibia breadth | breadth of metatibia, upper hind aspect | *70x |
| tib3.1 | metatibia length | length of metatibia upper hind aspect | $30 \times$ |

graph (Rencher 2002: 398-399). We also plotted isosize against shape PCs, because the correlation of size with shape is a measure of the amount of allometry in the data. Two graphical tools, the PCA ratio spectrum and allometry ratio spectrum respectively, were also employed in some cases. Finally, we used the LDA ratio extractor to extract the best ratios, and calculated the standard distance as well as the measure $\delta$.

The R language and environment for statistical computing was used for data analysis (R Development Core Team 2013; version 3.0.1). For the above methods we employed slightly modified versions of the R-scripts provided by Baur and Leuenberger (2011, under "Supplementary material"). Scatterplots were generated with the package "ggplot2" (Wickham 2009).

## Taxonomic treatment, voucher and type specimens

For taxonomy and classification we followed Carpenter (1996b). Abbreviations used for specimen depositories and other institutions or private collections cited in this study are given in Table 3. Stack-photographs of mounted specimens were taken with a Keyence VHX-2000 digital microscope at the NMBE. All known Polistes collections in Switzerland (CH), as well as several collections elsewhere (Table 3), have been examined by one of the authors (RN). We also examined the relevant type material.

Table 3. Abbreviations of depositories (museums and private collections) and other institutions. " CH " means Switzerland.

| abbreviation | full name |
| :--- | :--- |
| museums and other institutions |  |
| AMNH | American Museum of Natural History, New York, USA |
| BNM | Bündner Naturmuseum, Chur, CH |
| CSCF | Swiss Biological Records Center, Neuchâtel, CH |
| ETHZ | Eidgenössische Technische Hochschule, Zürich, CH |
| FMLT | Fundación Miguel Lillo, Tucumán, Argentina |
| HNHM | Hungarian Natural History Museum, Budapest, Hungary |
| HUMCZ | Harvard University Museum of Comparative Zoology, Cambridge, MA, USA |
| LSL | Linnean Society of London, GB |
| MACN | Museo Argentino de Ciencias Naturales, Buenos Aires, Argentina |
| MCHNS | Musée cantonal d'histoire naturelle, Sion, CH |
| MCSNL | Museo cantonale di storia naturale, Lugano, CH |
| MCSNV | Museo Civico di Storia Naturale, Verona, Italy |
| MFNB | Museum für Naturkunde, Berlin, Germany |
| MHNF | Musée d'histoire naturelle, Fribourg, CH |
| MHNG | Muséum d'histoire naturelle de la Ville de Genève, CH |
| MHNL | Museo de Historia Natural, Lima, Peru |
| MHNN | Muséum d'histoire naturelle de Neuchâtel, CH |
| MMML | Mĕstské muzeum Mariánské Lázĕ, Czech Republic |
| MNHN | Muséum national d'histoire naturelle, Paris, France |
| MSNM | Museo Civico di Storia Naturale, Milano, Italy |
| MSNUF | Museo di Storia Naturale dell'Università, Firenze, Italy |
| MSNV | Museo di Storia Naturale, Venezia, Italy |
| MZL | Musée cantonal de zoologie, Lausanne, CH |
| NBC | Naturalis Biodiversity Center, Leiden, Netherlands |
| NHMB | Naturhistorisches Museum Basel, CH |
| NHMW | Naturhistorisches Museum Wien, Austria |
| NMBE | Naturhistorisches Museum der Burgergemeinde Bern, CH |
| NML | Natur-Museum Luzern, CH |
| NMSO | Naturmuseum Solothurn, CH |
| NMTG | Naturmuseum Thurgau, Frauenfeld, CH |
| NMW | Naturmuseum Winterthur, CH |
| SDEI | Senckenberg Deutsches Entomologisches Institut, Müncheberg, Germany |
| ZIN | Zoological Institute of the Russian Academy of Sciences, Saint Petersburg, Russia |
| ZMUZ | Zoologisches Museum der Universität Zürich, CH |
| ZSM | Zoologische Staatssammlung München, Germany |

Private collections

| AK | Albert Krebs |
| :--- | :--- |
| AR | André Rey |
| CM | Christian Monnerat |
| CSE | Christian Schmid-Egger |
| ES | Erwin Steinmann |
| FA | Felix Amiet |
| GA | Georg Artmann-Graf |
| HT | Hansueli Tinner |
| IK | Igor Kramer |


| IS | Irene Salzmann |
| :--- | :--- |
| JF | Jakob Forster |
| JS | Jan Smit |
| LD | Libor Dvořák |
| MH | Mike Herrmann |
| PS | Peter Schär |
| RN | Rainer Neumeyer |
| WA | Werner Arens |
| WS | Wolfgang Schlaefle |
| YC | Yannick Chittaro |

## Data resources

The morphometric data underpinning the analyses reported in this paper as well as a series of images showing the exact character definitions are deposited in the Dryad Digital Repository at http://doi.org/10.5061/dryad.9b8tt.

## Results

## Molecular analyses

## a) Sequencing

Of the 99 ingroup specimens included, complete COX1 sequences were obtained for 96 specimens, and ITS sequences for 80 specimens (Table 1). This difference is due to 12 ITS sequences of $P$. nimpha that were polymorphic and excluded, as well as some specimens with degraded DNA, which could be sequenced for the shorter mitochondrial fragment but not for ITS1.

## b) COX1

Analyses of the COX1-sequences (Fig. 1) reveal that P. helveticus and P. bischoff represent two distinct, well-supported clades (Bootstrap support, hereafter BS, of 100 and $94 \%$, respectively). Sequences of all included specimens of $P$. bischoff, including the 10 specimens from Switzerland and one specimen from Corsica, were absolutely identical (genetic distance of 0 ); similarly, sequences of the 20 specimens of $P$. helveticus were identical. The genetic distance between these two clades was $2.6 \%$. The relationship between these two clades, as well as the relationships among the species of the gallicus-group, were not resolved.

More generally, most OTUs included in this study were recovered as monophyletic with high bootstrap support $>90 \%$, with the exception of $P$. dominula. Sequences for this OTU formed two well-supported clades (see below). The two specimens identified as Polistes gallicus by Arens (2011) did not appear closely related to other specimens of $P$. gallicus. For this reason, this taxon is simply referred to as Polistes sp. aff. gallicus.

Maximal within-OTU genetic distances were $0.2 \%$ for $P$. associus, $0.3 \%$ for $P$. sulcifer, $1.0 \%$ for $P$. bellenicus and for $P$. gallicus, $1.06 \%$ for $P$. sp. aff. gallicus, and $1.4 \%$ for


Figure I. Phylogenetic tree based on maximum likelihood analysis of sequences of the mitochondrial gene cytochrome oxidase 1 (COX1); numbers shown at nodes are bootstrap values based on 1000 bootstrap replicates performed in RAxML v.7.0.4. Branches interrupted by an oblique line have been shortened for better graphic representation.
P. biglumis. Within OTU-distances were higher for $P$. nimpha ( $2.4 \%$ ) and especially for $P$. dominula (up to 4.9\%; see below). For $P$. nimpha, although two weakly supported clades are revealed within this OTU (Fig. 1), the ranges of distance within ( $0-0.6 \%$ and $0-0.7 \%$ ) and between these clades ( $0.4-2.4 \%$ ) overlapped. In contrast, sequences for $P$. dominula formed two distinct clades that did not overlap. All sequences within the first clade were identical, thus the distance within this clade was equal to 0 . In the second clade, the


Figure 2. Phylogenetic tree based on maximum likelihood analysis of sequences of the nuclear marker ITS1; numbers shown at nodes are bootstrap values based on 1000 bootstrap replicates performed in RAxML v.7.0.4. Branches interrupted by an oblique line have been shortened for better graphic representation.
distances ranged from 0 to $0.67 \%$; the distances between these two clades were between 3.6 and $4.9 \%$. These two clades were weakly associated with geographic location: specimens originating from western Switzerland (Geneva, Valais and one location in Vaud) and from one site close to Zurich formed one clade, whereas specimens originating from the Grisons, from one location in Vaud and from the southern parts of the canton of Zurich formed the other clade; specimens from one locality in Zurich were distributed in both clades.

The minimal distance between two OTUs was $2.6 \%$, observed between $P$. helveticus and $P$. bischoff, as indicated above, as well as between the two included social parasites, $P$. semenowi and $P$. sulcifer.

## c) ITS1

Analyses of ITS1 (Fig. 2) again strongly suggest that P. helveticus and $P$. bischoffi represent two distinct, well supported clades (both with BS of 95\%). Sequences for all of the eleven specimens of $P$. bischoffi, including one specimen from Corsica, were identical; within $P$. helveticus, the genetic distance was $0.17 \%$ due to one single polymorphic site. The genetic distances between both clades were between $2.23 \%$ and $2.37 \%$. The relationship between these two species, as well as the relationships among the different species of the gallicus-group, were not resolved.

All other OTUs were recovered as well supported clades, with bootstrap supports $>85 \%$ (Fig. 2). No sequence of ITS1 could be obtained for the two specimens of $P$. sp. aff. gallicus from Greece. The two clades observed in analyses of the mitochondrial marker in $P$. dominula were not recovered in analyses of ITS1, although maximal within-OTU distances were comparatively high for this OTU ( $0.77 \%$ ). However, no distance correlation between ITS1 and COX1 was observed; for example, some specimens exhibiting high mitochondrial distances (eg, numbers 5 and 43) had identical ITS1 sequences. Other within-OTU genetic distances were as follows: $0 \%$ for P. sulcifer, P. associus and P. gallicus; $0.24 \%$ for $P$. biglumis; $0.32 \%$ for $P$. bellenicus.

The smallest interspecific distance in ITS1 sequences was $0.8 \%$, between $P$. biglumis and $P$. hellenicus; the maximal distance in our ingroup was $11.5 \%$, observed between P. nimpha and P. biglumis. The minimum distance between P. bischoff and any other OTU was $1.72 \%$, between bischoffi and P. biglumis.

## Multivariate ratio analysis (MRA) of the gallicus-group

As mentioned above in material and methods, we restricted the MRA to the five OTUs of the gallicus-group (s. Table 4 for an overview of measurements). We first performed a shape PCA to see how well the monophyletic OTUs recovered by molecular analyses (Figs 1 and 2) are supported by morphometric variation. A PCA is convenient because it does not require a priori assignment of OTUs to particular groups but assumes instead that all OTUs belong to one single group. A PCA thus avoids circular reasoning with respect to particular groupings (see Peters and Baur 2011). According to the scree graph (not shown), only the first and second shape PC were relevant, comprising more than $60 \%$ of the total variation. Scatterplots of the two axes gave a very similar result for both sexes (Figs 3a, b). P. biglumis was clearly separable from the other species along the first shape PC. The other OTUs were much closer, with $P$. bischoffi and $P$. helveticus still being rather distinct. The ranges of the two remaining OTUs, P. gallicus and P. hellenicus, were entirely overlapping. A scatterplot of isosize and the first shape PC revealed a strong correlation between size and shape (Figs 3c, d). This was mainly caused by the presence of P. biglumis, which was clearly the largest OTU in both sexes. The others were largely overlapping in their size ranges.

As mentioned in the introduction, two of the main target OTUs of our study, $P$. bischoffi and $P$. helveticus, are separated from the others by a reduced epicnemial carina.

Table 4. Summary of measurements (in $\mu \mathrm{m}$ ) used for morphometric analysis. For abbreviations, see table 1 .

| biglumis |  |  |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | females $\mathrm{n}=19$ |  |  |  | males $\mathrm{n}=20$ |  |  |  |
|  | Min | Max | Mean | SD | Min | Max | Mean | SD |
| cly.b | 1286 | 1586 | 1453.4 | 84.23 | 1157 | 1371 | 1241.4 | 52.62 |
| eye.d | 1540 | 1880 | 1704.2 | 98.79 | 1480 | 1760 | 1602 | 65.82 |
| eye.h | 2033 | 2500 | 2268.4 | 131.22 | 2067 | 2433 | 2280 | 80.5 |
| flgfirst.l | 829 | 1000 | 932.3 | 48.06 | 1000 | 1129 | 1081.4 | 37.11 |
| flglast.b | 286 | 343 | 315 | 15.95 | 157 | 186 | 172.9 | 9.15 |
| flglast. 1 | 300 | 393 | 334.6 | 22.75 | 293 | 421 | 364.3 | 37.08 |
| hea.b | 2933 | 3500 | 3238.6 | 175.07 | 3067 | 3533 | 3261.7 | 98.1 |
| hea.h | 2600 | 3033 | 2805.3 | 136.2 | 2467 | 2833 | 2643.3 | 75.78 |
| lof. 1 | 1580 | 1920 | 1751.6 | 94.83 | 1540 | 1840 | 1695 | 59.78 |
| msp. 1 | 371 | 514 | 445.5 | 34.85 | 371 | 436 | 403.6 | 22.53 |
| ool. 1 | 514 | 629 | 578.6 | 31.59 | 471 | 571 | 529.3 | 29.03 |
| pol. 1 | 264 | 379 | 325.2 | 33.53 | 279 | 386 | 326.4 | 22.72 |
| scp.b | 379 | 486 | 444 | 28.3 | 450 | 564 | 512.1 | 26.74 |
| scp.l | 1157 | 1400 | 1281.2 | 71.75 | 1129 | 1314 | 1202.1 | 49.55 |
| tib3.b | 507 | 650 | 585 | 40.05 | 521 | 614 | 577.9 | 29.3 |
| tib3.1 | 2900 | 3767 | 3387.7 | 250.73 | 3000 | 3733 | 3428.3 | 163.04 |
| bischoffi |  |  |  |  |  |  |  |  |
|  | females $\mathrm{n}=46$ |  |  |  | males $\mathrm{n}=13$ |  |  |  |
|  | Min | Max | Mean | SD | Min | Max | Mean | SD |
| cly.b | 1057 | 1386 | 1216.5 | 90.3 | 943 | 1100 | 983.5 | 44.74 |
| eye.d | 1300 | 1640 | 1453.9 | 80.76 | 1280 | 1480 | 1352.3 | 57.47 |
| eye.h | 1833 | 2333 | 2069.6 | 135.32 | 1900 | 2167 | 2056.4 | 87.54 |
| flgfirst.1 | 671 | 886 | 781.1 | 53.03 | 871 | 1000 | 944 | 30.56 |
| flglast. ${ }^{\text {b }}$ | 257 | 314 | 286.5 | 14.85 | 150 | 171 | 158.2 | 7.05 |
| flglast. 1 | 279 | 386 | 320.2 | 18.83 | 400 | 471 | 425.3 | 23.26 |
| hea.b | 2533 | 3167 | 2842 | 163.52 | 2667 | 3033 | 2864.1 | 100.43 |
| hea.h | 2167 | 2767 | 2447.8 | 152.45 | 2167 | 2433 | 2305.1 | 81.47 |
| lof. 1 | 1340 | 1720 | 1516.1 | 102.99 | 1460 | 1620 | 1520 | 48.99 |
| msp. 1 | 250 | 350 | 289.4 | 22.57 | 171 | 207 | 187.4 | 10.97 |
| ool.1 | 429 | 529 | 472.8 | 26.92 | 379 | 479 | 417 | 28.35 |
| pol. 1 | 264 | 357 | 321.9 | 21.63 | 293 | 364 | 319.8 | 20.24 |
| scp.b | 314 | 414 | 365.1 | 27.32 | 379 | 450 | 416.5 | 20.07 |
| scp. 1 | 986 | 1271 | 1113.7 | 70.5 | 943 | 1100 | 1041.8 | 46.46 |
| tib3.b | 393 | 600 | 486 | 46.19 | 429 | 500 | 470.9 | 24.99 |
| tib3.1 | 2600 | 3633 | 3072.5 | 256.69 | 2767 | 3233 | 3061.5 | 156.26 |
| gallicus |  |  |  |  |  |  |  |  |
|  | females $\mathrm{n}=42$ |  |  |  | males $\mathrm{n}=25$ |  |  |  |
|  | Min | Max | Mean | SD | Min | Max | Mean | SD |
| cly.b | 1143 | 1471 | 1290.5 | 74.11 | 900 | 1186 | 1026.3 | 59.29 |
| eye.d | 1420 | 1720 | 1560.5 | 73.58 | 1200 | 1560 | 1419.2 | 75.38 |
| eye.h | 1900 | 2533 | 2134.1 | 125.26 | 1833 | 2267 | 2064 | 118.21 |
| flgfirst. 1 | 714 | 929 | 795.6 | 46.85 | 886 | 1114 | 975.4 | 48.36 |
| flglast.b | 271 | 336 | 303.2 | 14.13 | 136 | 179 | 160.6 | 12.39 |
| flglast. 1 | 286 | 357 | 313.6 | 18.92 | 329 | 457 | 385.4 | 33.02 |


| hea.b | 2733 | 3400 | 3017.5 | 149.3 | 2633 | 3233 | 2921.3 | 149.96 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| hea.h | 2333 | 2900 | 2556.3 | 134.24 | 2167 | 2733 | 2401.3 | 122.66 |
| lof.l | 1420 | 1820 | 1583.8 | 89.14 | 1420 | 1820 | 1575.2 | 87.99 |
| msp.l | 293 | 393 | 333.3 | 24.93 | 186 | 307 | 243.1 | 27.85 |
| ool.l | 457 | 571 | 512.4 | 32.02 | 357 | 521 | 450.3 | 28.31 |
| pol.l | 279 | 371 | 333.3 | 21.32 | 271 | 371 | 308.9 | 27.53 |
| scp.b | 343 | 436 | 383.7 | 22.1 | 379 | 471 | 428 | 22.77 |
| scp.l | 1057 | 1343 | 1172.4 | 61.21 | 914 | 1171 | 1054.3 | 62.13 |
| tib3.b | 471 | 607 | 525.7 | 37.5 | 436 | 600 | 493.1 | 37.6 |
| tib3.l | 2767 | 3733 | 3211.9 | 236.94 | 2600 | 4000 | 3212 | 269.92 |

bellenicus

|  | females $\mathrm{n}=21$ |  |  |  |  | males $\mathrm{n}=9$ |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | Min | Max | Mean | SD | Min | Max | Mean | SD |  |
| cly.b | 1171 | 1457 | 1313.6 | 87.19 | 957 | 1057 | 1014.3 | 34.99 |  |
| eye.d | 1440 | 1720 | 1568.6 | 92.43 | 1280 | 1540 | 1422.2 | 98.71 |  |
| eye.h | 2000 | 2400 | 2188.9 | 143.89 | 1933 | 2200 | 2055.6 | 92.8 |  |
| flgfirst.l | 743 | 929 | 831.3 | 51.63 | 871 | 986 | 941.3 | 40.05 |  |
| flglast.b | 279 | 336 | 299 | 15.2 | 143 | 171 | 155.6 | 8.58 |  |
| flglast.l | 271 | 371 | 314.3 | 27.94 | 364 | 414 | 391.3 | 18.48 |  |
| hea.b | 2767 | 3300 | 3036.5 | 172.85 | 2600 | 2967 | 2837 | 112.35 |  |
| hea.h | 2367 | 2867 | 2603.2 | 158.43 | 2200 | 2467 | 2377.8 | 83.33 |  |
| lof.1 | 1460 | 1820 | 1613.3 | 113.37 | 1460 | 1640 | 1566.7 | 58.31 |  |
| msp.l | 279 | 400 | 341.5 | 34 | 179 | 257 | 219 | 22.87 |  |
| ool.l | 457 | 564 | 517.7 | 32.62 | 414 | 514 | 462.7 | 30.88 |  |
| pol.1 | 271 | 357 | 322.8 | 20.78 | 286 | 336 | 302.4 | 20.52 |  |
| scp.b | 364 | 436 | 396.9 | 18.98 | 379 | 443 | 420.6 | 22.99 |  |
| scp.l | 1086 | 1286 | 1181 | 72.19 | 957 | 1100 | 1042.9 | 40.41 |  |
| tib3.b | 500 | 614 | 544.6 | 32.25 | 464 | 521 | 496 | 20.55 |  |
| tib3.l | 3033 | 3767 | 3360.3 | 246.45 | 3033 | 3333 | 3225.9 | 124.47 |  |

helveticus

|  | females $\mathrm{n}=34$ |  |  |  |  |  | males n=27 |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | Min | Max | Mean | SD | Min | Max | Mean | SD |  |  |
| cly.b | 1071 | 1457 | 1254.2 | 83.36 | 914 | 1057 | 994.2 | 37.64 |  |  |
| eye.d | 1420 | 1780 | 1538.8 | 79.99 | 1280 | 1600 | 1425.2 | 71.6 |  |  |
| eye.h | 1800 | 2333 | 2045.1 | 129.2 | 1800 | 2133 | 1998.8 | 82.42 |  |  |
| flgfirst.l | 657 | 871 | 780.7 | 49.47 | 857 | 986 | 928 | 33.03 |  |  |
| flglast.b | 264 | 321 | 295 | 16.82 | 129 | 193 | 161.6 | 12.07 |  |  |
| flglast.l | 300 | 407 | 327.9 | 23.81 | 279 | 457 | 346.8 | 37.76 |  |  |
| hea.b | 2567 | 3233 | 2866.7 | 155.27 | 2633 | 3033 | 2893.8 | 90.13 |  |  |
| hea.h | 2267 | 2833 | 2521.6 | 147 | 2133 | 2467 | 2355.6 | 71.61 |  |  |
| lof.l | 1380 | 1780 | 1552.4 | 100.79 | 1400 | 1620 | 1547.4 | 54.95 |  |  |
| msp.l | 293 | 429 | 357.4 | 29.92 | 214 | 293 | 249.5 | 20.2 |  |  |
| ool.1 | 450 | 607 | 515.1 | 28.07 | 386 | 536 | 450 | 31.13 |  |  |
| pol.l | 293 | 414 | 348.3 | 27.75 | 279 | 379 | 328.6 | 24.82 |  |  |
| scp.b | 343 | 457 | 387.6 | 31.52 | 386 | 464 | 430.2 | 16.38 |  |  |
| scp.l | 1000 | 1300 | 1129.8 | 70.63 | 943 | 1086 | 1028 | 33.26 |  |  |
| tib3.b | 421 | 600 | 506.1 | 48.06 | 429 | 536 | 487.3 | 27.83 |  |  |
| tib3.l | 2667 | 3567 | 3049 | 223.27 | 2800 | 3300 | 3080.2 | 142.1 |  |  |



Figure 3. Shape PCA of all five OTUs of the Polistes gallicus-group. a, b Scatterplot of first against second shape PC a females $\mathbf{b}$ males $\mathbf{c}, \mathbf{d}$ Scatterplot of isosize against first shape PC $\mathbf{c}$ females $\mathbf{d}$ males. Symbols: orange circles $=$ biglumis, purple triangles $=$ bischoff, green diamonds $=$ gallicus, blue rectangles $=$ hellenicus, red dots $=$ helveticus. In parentheses, the variance explained by each shape PC. Encircled upper case letters show the position of the type specimens of various nominal taxa: (A) P. bimaculatus pamirensis Zirngiebl, 1955, syntypes; (B) P. bimaculatus nigrinotum Zirngiebl, 1955, holotype; (C) P. bischoff Weyrauch, 1937, neotype; (D) P. foederatus obscuricornis Mader, 1936, syntypes; (E) P. omissus ordubadensis Zirngiebl, 1955, holotype; (F) P. omissus kaszabi Giordani Soika, 1970, holotype; (G) P. foederatus albellus Giordani Soika, 1976, paratype; (H) P. helveticus Neumeyer sp. n., holotype; (I) P. dubius Kohl, 1898, lectotype; (J) P. foederatus Kohl, 1898, holotype; (K) P. gallicus mongolicus Buysson, 1911, syntype; (L) P. hellenicus Arens, 2011, holotype.

We therefore conducted a shape PCA including only these two OTUs for examining their morphometric differences. Only the first shape PC was informative and was plotted against isosize to evaluate the amount of allometric variation in the data (Fig. 4). Both sexes were well differentiated by the first shape PC. Furthermore, females of $P$. helveticus were very slightly larger than those of $P$. bischoffi (4a), whereas males were entirely overlapping in the size range (Fig. 4b).


Figure 4. Scatterplot of first shape PC against isosize for comparison of Polistes bischoff with $P$. helveticus: $\mathbf{a}$ females $\mathbf{b}$ males. Symbols: purple triangles $=$ bischoff, red dots $=$ helveticus; closed symbols $=$ specimens identified by genetic clustering and morphological characters; open symbols = specimens identified by morphological characters only. In parentheses the variance explained by the first shape PC.

Polistes bischoffi-helveticus, females

a

Polistes bischoffi-helveticus, males


Figure 5. PCA ratio spectrum (blue) and allometry ratio spectrum (green) of a comparison of Polistes bischoff with $P$. helveticus. a females $\mathbf{b}$ males. Horizontal bars in PCA and allometry ratio spectrum represent $68 \%$ bootstrap confidence intervals based on 1000 replicates.

To interpret the first shape PC, the PCA ratio spectrum was plotted (Fig. 5, graph with blue bars). In a PCA ratio spectrum, only ratios calculated with variables lying at the opposite ends of the spectrum are relevant for a particular shape PC (Baur and Leuenberger 2011). In a similar manner, the most allometric ratios are found in an allometry ratio spectrum (Fig. 5, graph with green bars). For females (Fig. 5a) the PCA ratio spectrum was dominated by ratios msp.l : eye.h, msp.l : tb3.l, and msp.l :

Table 5. Best ratios found by the LDA ratio extractor for separating various groupings of Polistes. Asterisks mark those comparisons, where the groups have very little or no overlap and the best ratios thus were eligible for use in the identification key.

| females |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: |
| group comparison | best ratio | range group 1 | range group 2 | standard distance | $\delta$ |
| biglumis-rest* | msp.l : pol.1 | 1.22-1.76 | 0.79-1.24 | 4.62 | 0.29 |
| belveticus-bischoff** | tib3.1: msp.1 | 7.61-9.33 | 9.57-11.5 | 6.19 | 0.10 |
| belveticus-gallicus | hea.b : msp.l | 7.29-8.80 | 8.23-10.08 | 4.34 | 0.05 |
| belveticus-hellenicus | tib3.1: msp.1 | 7.61-9.33 | 9.00-11.13 | 4.72 | 0.09 |
| bischoff-gallicus | flglast.1 : msp.l | 1.00-1.24 | 0.84-1.07 | 4.07 | 0.19 |
| bischoff_-hellenicus | flglast.1 : msp. 1 | 1.00-1.24 | 0.74-1.11 | 4.25 | 0.22 |
| males |  |  |  |  |  |
| group comparison | best ratio | range group 1 | range group 2 | standard distance | $\delta$ |
| biglumis-rest* | lof.l : cly.b | 1.29-1.45 | 1.46-1.69 | 8.36 | 0.31 |
| helveticus-bischoff** | flglast.1: msp.l | 1.08-1.72 | 1.93-2.75 | 6.82 | 0.08 |
| belveticus-gallicus | flglast.1: pol.1 | 0.83-1.32 | 1.09-1.55 | 3.54 | 0.09 |
| helveticus-hellenicus | flglast.1: msp. 1 | 1.08-1.72 | 1.44-2.16 | 5.92 | 0.01 |
| bischooff-gallicus | flglast.1 : msp.1 | 1.93-2.75 | 1.28-2.21 | 3.50 | 0.18 |
| bischoff_hellenicus* | hea.b : hea.h | 1.21-1.29 | 1.15-1.21 | 6.27 | 0.10 |

flgfirst.l; for males (Fig. 5b) only a single ratio was most important, msp.l : flglast.l. The same ratio was also the most allometric (though both variables showed broad confidence intervals, see allometry ratio spectrum for males, Fig. 5b), whereas for females the dominating ratios were not among the most allometric ones (Fig. 5a). This result was in accordance with the general observation that allometric variation played a minor role in distinguishing the two groups, as they were of comparable size (compare Fig. 4).

The LDA ratio extractor is a tool for finding the best discriminating ratios for use in identification keys and diagnoses (see Baur and Leuenberger 2011). In contrast to a PCA, group membership must be specified beforehand. The results are compiled in Table 5 showing various contrasts, listed by sex. Generally, males were more distinct than females, as the groups were more widely separated in their ranges and the standard distances were on average higher, though overlapping (3.50-8.36 for males versus 4.07-6.19 for females). The ranges of two female comparisons (biglumis-rest, helveticus-bischoffi) were more or less distinct, for males a third one could be added (bischoffi-hellenicus). Ratios that separated the groups well were used for the key and diagnoses (see below). For both sexes, $\delta$ (a measure of how well shape discriminates in comparison with size) was always relatively close to zero (0.01-0.31), indicating that separation was mainly due to shape rather than size.

## Taxonomic treatment

a) Status of OTUs

Our molecular and morphometric analyses clearly revealed that all operational taxonomic units (hitherto called OTUs) formed well-supported taxonomic units (i.e., species). We can thus confidently conclude that the three species examined in this study, P. bischoffi, P. gallicus, and P. helveticus sp. n., represent valid species.

## b) Diagnoses and descriptions of species

The following section provides information on all five species of the gallicus-group, as these can most easily be confused with each other, including the two main target taxa, P. bischoffi and P. helveticus sp. n.

## Polistes biglumis (Linnaeus, 1758)

http://species-id.net/wiki/Polistes_biglumis
Vespa biglumis Linnaeus, 1758: 573 - Holotype female (LSL), designation by Day (1979), type locality Europe (not examined)

Vespa rupestris Linnaeus, 1758: 573 - Holotype male (LSL), designation by Day (1979), type locality Sweden (not examined)

Vespa bimaculata Geoffroy in Fourcroy 1785: 433 - Holotype female (type lost; see Blüthgen 1961: 54), type locality near Paris, France
Polistes geoffroyi Lepeletier \& Serville, 1825: 173 - Syntypes males, females (depository unknown), type locality France
Polistes dubius Kohl, 1898: 90 - Lectotype male (NHMW), designated by Blüthgen (1943: 128), type locality Brühl, Austria (examined by RN)
Polistes kohli Dalla Torre, 1904: 70 - Replacement name for Polistes dubius Kohl, 1898, nec Saussure, 1867
Polistes bimaculatus pamirensis Zirngiebl, 1955: 381-383, 385 - Syntypes 4 females (ZSM), type locality "Umss-Tugai", probably in the area of eastern Uzbekistan to southwestern Tadjikistan (examined by RN)
Polistes bimaculatus nigrinotum Zirngiebl, 1955: 381-383, 385 - Holotype female (ZSM), type locality Althegnenberg, Germany (examined by RN)

Diagnosis. Relatively large, dark species with pedicel and flagellum dorsally black in both sexes.

Females: Epicnemial carina pronounced (Fig. 12a). Hypopygium black. Metacoxa black. Mesoscutum black. Propodeum black laterally, occasionally with small yellow spot. Clypeus breadth : malar space 3.06-3.58; head breadth : malar space 6.81-8.09;
malar space : lateral ocelli distance 1.22-1.76; metatibia length : malar space 6.968.56; terminal flagellomere length : malar space 0.64-0.87.

Males: Gena in dorsal view convex (Fig. 12l). Epicnemium and mesosternum yellow. Head breadth : head height 1.18-1.28; lower face : clypeus breadth 1.29-1.45; terminal flagellomere length : lateral ocelli distance $0.85-1.38$; terminal flagellomere length : malar space 0.72-1.07; terminal flagellomere length : terminal flagellomere breadth 1.64-2.68.

Comments. The holotype of Vespa biglumis Linnaeus, 1758, presently held at the Linnean Society of London, is not available for loan. We have, however, examined pictures online (http://linnean-online.org/16745/). Although no clear epicnemial carina is recognizable from the picture due to the condition of the specimen, the pubescence on the mesoscutum appears too long for $P$. helveticus sp. $n$. Therefore, we have no reason to question the current concept of $P$. biglumis.

Similarly, we have examined pictures (http://linnean-online.org/16772/) of the holotype (LINN 2807) of Vespa rupestris Linnaeus, 1758, also held at the Linnean Society of London and unavailable for loan. The genae of this male specimen are clearly convex in dorsal view (Fig. 12l), excluding any confusion with $P$. helveticus sp. n. or P. bischoff.

The holotype of Vespa bimaculata Geoffroy in Fourcroy, 1785 is missing (Blüthgen 1961: 54), as are the syntypes of Polistes geoffroyi Lepeletier \& Serville, 1825. According to the original descriptions both taxa seem to refer to dark individuals, but since no epicnemial carina is mentioned, a synonymy with $P$. helveticus sp. n. can neither be excluded nor proved.

The lectotype of Polistes dubius Kohl, 1898 was examined; we did not detect any characters allowing separation from P. biglumis. This view is also supported by our morphometric analyses (Fig. 3b, d [I]), which revealed that the lectotype of P. dubius does not plot far away from other males of $P$. biglumis. In any case it is a male with convex genae (Fig. 12l), making any confusion with the otherwise similarily colored male of $P$. helveticus $\mathrm{sp} . \mathrm{n}$. impossible.

We have seen three (ZSM-HYM-000006, ZSM-HYM-000007, ZSM-HYM-000009) of four syntypes of Polistes bimaculatus pamirensis Zirngiebl, 1955. Although they are dark females, occasionally with the entire mandible (ZSM-HYM-000007) or the apical part of the clypeus (ZSM-HYM-000006, ZSM-HYM-000007) black, the flagellum is not dark even on its dorsal side. The epicnemial carina is very pronounced in all three specimens, excluding confusion with $P$. helveticus sp. n. or P. bischoffi. However, morphology as well as morphometry (Fig. 3a, c [A]) cast doubt on whether this taxon is conspecific with $P$. biglumis. More material and further studies are needed to elucidate the status of this taxon.

The holotype (ZSM-HYM-000008) of Polistes bimaculatus nigrinotum Zirngiebl, 1955 is a very dark female; the apical part of the clypeus is entirely black and there is only a very small yellow spot on the mandible. The epicnemial carina is distinct, excluding confusion with $P$. helveticus sp . n . We see no trait distinguishing this specimen from P. biglumis, a view supported by our morphometric analysis (Fig. 3a, c [B]).

Material examined. 1 § (Lectotype of $P$. dubius): AUSTRIA, LOWER AUSTRIA, Brühl, 22 Aug 1883, Franz Friedrich Kohl det., NHMW coll.; 2 Ø (RN0123, RN0124): CROATIA, ISTRIA, Vela Učka, $45^{\circ} 18^{\prime} 25.7^{\prime \prime} \mathrm{N}, 14^{\circ} 11^{\prime} 40.4^{\prime \prime} \mathrm{E}, 824 \mathrm{~m}$, 27 Jul 2012, karst mountain range, Rainer Neumeyer leg., RN coll.; $1 \sigma^{\top}$ (RN0224): GERMANY, BADEN-WÜRTTEMBERG, Pullendorf, 13 Jul 2009, railroad area, Mike Herrmann leg., MH coll.; 1 q (Holotype of bimaculatus nigrinotum: ZSM-HYM-000008): BAVARIA, Althegnenberg, 19-20 Jul 1946, Heinz Freude leg., ZSM coll.; 1 § (RN0231): SWITZERLAND, CANTON BASLE-CITY, Basel, Badischer Bahnhof, $47^{\circ} 34^{\prime} 50.12$ "N, $07^{\circ} 36^{\prime} 07.63^{\prime \prime} \mathrm{E}, 255 \mathrm{~m}, 18$ Aug 1995, railroad area, Rainer Neumeyer leg., RN coll.; 1 ő (RN0247): CANTON GRISONS, Buseno, Monti di San Carlo, 1200 m, 09 Jul 1942, Adolf Nadig leg., ETHZ coll.; 1 ㅇ (RN0249): Davos, Züge, 1500 m, 27 Aug 1931, Johann Peter Wolf leg., ETHZ coll.; 1 \& (RN0239): Feldis/Veulden, 21 Sep 1935, Adolf Nadig leg., ETHZ coll.; 1 § (RN0248): 25 Aug 1944, Adolf Nadig leg., ETHZ coll.; 1 \& (RN0246): Ftan, 1610 m, 21 Jul 1994, Bernhard Merz leg., ETHZ coll.; 1 \& (RN0229): KlostersSerneus, Boschga, $46^{\circ} 52^{\prime} 52.20 " \mathrm{~N}, 09^{\circ} 52^{\prime} 14.34^{\prime \prime} \mathrm{E}, 1060 \mathrm{~m}, 01$ May 1993, montane meadow, Rainer Neumeyer leg., RN coll.; 2 § (RN0250, RN0258): Ramosch, 07 Sep 1963, Willi Sauter leg., ETHZ coll.; 1 q (RN0238): Sumvitg, Rabius, 24 Jun 1934, Adolf Nadig leg., ETHZ coll.; 1 § (RN0255): Val Müstair, Lü, 13 Aug 1935, Adolf Nadig leg., ETHZ coll.; 1 Q (RN0028): Val Müstair, Müstair: Munt Masaun, $46^{\circ} 37^{\prime} 02.21^{\prime \prime} \mathrm{N}, 10^{\circ} 25^{\prime} 56.36^{\prime \prime} \mathrm{E}, 1420 \mathrm{~m}, 13$ Aug 2011, rock steppe, Rainer Neumeyer leg., RN coll.; $1 \sigma^{\top}$ (RN0027): Val Müstair, Sta. Maria: Costas, $46^{\circ} 36^{\prime} 25.78$ "N, $10^{\circ} 25^{\prime} 29.37^{\prime \prime} \mathrm{E}, 1350 \mathrm{~m}, 13$ Aug 2011, berm, Rainer Neumeyer leg., RN coll.; 1 q (RN0029): Val Müstair, Tschierv: God da Munt, $46^{\circ} 37^{\prime} 48.79^{\prime \prime} \mathrm{N}, 10^{\circ} 20^{\prime} 42.11^{\prime \prime} \mathrm{E}$, 1790 m, 14 Aug 2011, clear larch wood, Rainer Neumeyer leg., RN coll.; $1 q+1$ § (RN0021, RN0087): Hansueli Tinner leg., RN coll.; 1 q (RN0243): Zuoz, 1800 m, 09 Sep 1966, Paul Bovey leg., ETHZ coll.; 1 ő (RN0233): CANTON ST. GALLEN, Pfäfers, Bläser Berg, $46^{\circ} 57^{\prime} 24.76^{\prime \prime} \mathrm{N}, 09^{\circ} 29^{\prime} 51.11^{\prime \prime} \mathrm{E}, 1500 \mathrm{~m}, 23$ Aug 1994, blowdown, Peter Duelli leg., RN coll.; 1 § (RN0256): Pfäfers, 20 Sep 1955, Hedwig Huber leg., ETHZ coll.; $1 \lesssim$ (RN0254): Walenstadt, Steinbruch Engen, 04 Sep 1997, quarry, Andreas Müller leg., ETHZ coll.; $1 \sigma^{\top}$ (RN0232): CANTON SCHAFFHAUSEN, Merishausen, Chörblihalde, $47^{\circ} 45^{\prime} 24.8^{\prime \prime} \mathrm{N}, 08^{\circ} 37^{\prime} 16.59^{\prime \prime} \mathrm{E}, 565 \mathrm{~m}, 25$ Aug 2004, hay meadow, Rainer Neumeyer leg., RN coll.; 1 q (RN0228): Merishausen, Laadel, $47^{\circ} 46^{\prime} 17.63^{\prime \prime} \mathrm{N}, 08^{\circ} 36^{\prime} 25.42 \mathrm{~L} \mathrm{E}, 620 \mathrm{~m}, 16$ Aug 2004, fallow meadow, Rainer Neumeyer leg., RN coll.; 1 q (RN0225): CANTON THURGOVIA, Herdern, $47^{\circ} 36^{\prime} 39.59^{\prime \prime} \mathrm{N}, 08^{\circ} 54^{\prime} 20.39^{\prime \prime} \mathrm{E}, 635 \mathrm{~m}$, Aug 2005, Mike Herrmann leg., MH coll.; 1 \& (RN0240): CANTON TICINO, Airolo, 04 Aug 1933, Adolf Nadig leg., ETHZ coll.; 1 \& (RN0227): Lavertezzo, Piano, $46^{\circ} 15^{\prime} 54.34^{\prime \prime N}, 08^{\circ} 49^{\prime} 15.23^{\prime \prime} \mathrm{E}, 589$ m, 01 Jun 2012, Yannick Chittaro leg., YC coll.; 1 § (RN0236): Prato Sornico, Lovalt, $46^{\circ} 23^{\prime} 20.36^{\prime \prime N}, 08^{\circ} 39^{\prime} 51.38^{\prime \prime} \mathrm{E}, 610 \mathrm{~m}, 24$ Jul 1994, riparian zones, Rainer Neumeyer leg., RN coll.; 1 q (RN0178): Prugiasco, San Carlo di Negrentino, $46^{\circ} 27^{\prime} 46.1^{\prime \prime} \mathrm{N}, 08^{\circ} 55^{\prime} 24.50^{\prime \prime} \mathrm{E}, 860 \mathrm{~m}, 20$ Aug 1993, montane meadow, Rainer Neumeyer leg., RN coll.; 1 ( q 0257): CANTON VALAIS, Ausserberg, Millachra,
$46^{\circ} 19^{\prime} 08.04$ "N, $07^{\circ} 50^{\prime} 14.02^{\prime \prime} \mathrm{E}, 1210 \mathrm{~m}, 09 \mathrm{Jul}$ 1998, Rainer Neumeyer leg., RN coll.; $1 \precsim$ (RN0234): 06 Sep 1998, Rainer Neumeyer leg., RN coll.; 1 Q (RN0244): Erschmatt, Rüemetschbodu, $46^{\circ} 19^{\prime} 35.42^{\prime \prime} \mathrm{N}, 07^{\circ} 41^{\prime} 44.68^{\prime \prime} \mathrm{E}, 1490 \mathrm{~m}, 18 \mathrm{Jul} 2003$, Alexandra Breitenstein leg., ETHZ coll.; 1 Ø (RN0252): Fiesch, 28 Jul 1942, Adolf Nadig leg., ETHZ coll.; $1 \sigma^{\Uparrow}$ (RN0253): Grimentz, 18 Aug 1941, Adolf Nadig leg., ETHZ coll.; $1 \circlearrowleft^{\top}$ (RN0235): Guttet-Feschel, 1300 m, 1993, Gerhard Bächli leg., RN coll.; 1 \& (RN0241): Ried-Brig, Berisal, 30 Jun 1919, anon. leg., ETHZ coll.; 1 (RN0226): Ried-Brig, Gantertal, $46^{\circ} 17^{\prime} 56.61^{\prime \prime} \mathrm{N}, 08^{\circ} 03^{\prime} 35.70^{\prime \prime} \mathrm{E}, 1420 \mathrm{~m}, 26$ Jun 2012, Yannick Chittaro leg., YC coll.; 3 P (Syntypes of bimaculatus pamirensis: ZSM-HYM-000006, ZSM-HYM-000007, ZSM-HYM-000009): UZBEKISTAN or TADJIKISTAN, "Umss-Tugai", 25 Jul 1928, Willi Rickmers leg., ZSM coll.

## Polistes bischoffi Weyrauch, 1937, rev. stat.

Polistes bischoffi Weyrauch, 1937: 274 - Neotype female (NMBE), present designation, type locality Galeria, Corsica, France
"Polistes cf. gallicus" - Neumeyer et al. (2011)
Type study. Polistes bischoffi was described by Weyrauch (1937: 274) in a mere footnote indicating neither the type material nor the type locality. Later, Weyrauch (1938: 277 ff .) gave a key to the Palearctic species of Polistes, including P. bischofff, but a more precise indication of the type material and the type locality is given only in Weyrauch (1939: 163), where a female from Macomer (Sardinia, Italy) is mentioned as the "type [Typus]". However, following article 74.5 of the ICZN (2012) this specimen is considered as a lectotype here. Unfortunately, this lectotype is lost (Blüthgen 1956: 85), as well as most paralectotypes from various localities (Italy, Malta, and Turkey; see Weyrauch 1939: 164), with the exception of two presumed paralectotypes that we were able to examine: a female (RN0287) from the Greek Island of Poros (see below, examined material), and a male (RN0325) from Glattbrugg in Switzerland. While the male from Glattbrugg clearly belongs to the dark (Fig. 10), northern (Fig. 11) taxon (Polistes helveticus sp. n.), the female from Poros belongs without any doubt to the southern (Fig. 11), bright (Fig. 6) taxon (Polistes bischoffi). Consequently, Weyrauch (1939) most likely considered both taxa as geografically separated color morphs of the same species. Evidence for this statement can be found in his redescription of $P$. bischoffe (Weyrauch 1939: 163 ff .), where he writes that the antenna is "dorsally blackened in the northern part of the species range [Fühler im Norden des Verbreitungsgebietes oberseits geschwärzt]".

It must be stressed that both taxa ( $P$. bischoffi, $P$. helveticus sp. n.) run to "bischoffi" in the keys of Weyrauch (1938: 277 ff.; 1939: 195 ff.). In more recent keys (Blüthgen 1961, Dvořák and Roberts 2006, Guiglia 1972, Mauss and Treiber 2004, Witt 2009) for Central Europe however, Polistes helveticus sp. n. would run to "bischoff", whereas Polistes bischoffi would run to "gallicus" due to the entirely bright flagellum.


Figure 6. Different aspects of a female (specimen RN0137) of Polistes bischoffi Weyrauch, 1937: a frontal view of head $\mathbf{b}$ lateral view of lower face with malar space and mandible $\mathbf{c}$ lateral view of head and mesosoma d lateral view of body e body from above. Arrows indicate the yellow patch on the lateral part of propodeum (c) and one of two yellow spots on the mesoscutum (e).

Unfortunately, the identity of the lost lectotype from Macomer (Sardinia, Italy) is unclear and can not be guessed from Weyrauch (1937, 1938, 1939). Therefore, the designation of a neotype is necessary for the clarification of the identity of Polistes bischofff. Our attempts to locate the lectotype in all institutions likely to host some of Weyrauch's material were unsuccessful (e.g.: MFNB, Michael Ohl, pers. comm.; MHNL, Claus Rasmussen, pers. comm.; FMLT, Emilia Perez, pers. comm.), and so were our attempts to locate any specimen of Polistes bischoffi from Sardinia, including during a field trip to Macomer in 2013. Consequently, we designate a female from Galeria on the island of Corsica (France), north of Sardinia, as the neotype of Polistes bischoff. Given that there is only a distance of 12 km between the two neighboring islands (Corsica, Sardinia), and that both of them share a similar fauna (Corti et al. 1999; Kwet 2005; Tolman and Lewington 1997), we are confident that this specimen matches the lost lectotype of Polistes bischoffi Weyrauch, 1937. In fact both, Corsica and Sardinia are probably located too far south to host the taxon called Polistes helveticus sp. n. here, since the southernmost individual (RN0378) of P. helveticus sp. n. that we are familiar with was found about 200 km north of the French Mediterranean coast (Fig. 11). Moreoever, the neotype is a well preserved female of the southern, light colored species (P. bischoff) that appears at the


Figure 7. Different aspects of a male (specimen RN0151) of Polistes bischoff Weyrauch, 1937: a frontal view of head $\mathbf{b}$ lateral view of lower face with malar space and mandible $\mathbf{c}$ dorsal view of body $\mathbf{d}$ lateral view of head, mesosoma, and base of metasoma $\mathbf{e}$ lateral view of body. The red arrow in $\mathbf{b}$ is pointing to the faint lateral ridge on clypeus. In $\mathbf{d}$ the white arrow is pointing to the yellow ventrolateral stripe of the pronotum, the red arrow to the lateral extension of terminal band on tergum 2 , the black arrow to a large yellow spot on sternum 2 and the blue arrow on the yellow basal band of sternum 3 .
center of the scatter of points in our morphometric analysis and clearly lies outside the area of overlap with P. gallicus (Fig. 3a, c [C]). Lastly, this specimen (RN0366) yielded high-quality DNA and could be included in our molecular analysis.

Diagnosis. Small and moderately bright species with flagellum on upper side bright yellow in both sexes (Figs 6a, 6c, 6d, 7a, 7b, 7d, 7e) or faintly darkened, especially in large females; pedicel and extreme base of flagellomere 1 always black on upper side.

Females: Epicnemial carina reduced (Figs 6c, 12b) or absent. Hypopygium black. Metacoxa usually black, seldom spotted yellow on upper side. Mesoscutum usually black, seldom with minute pair of yellow spots (Fig. Ge; arrow). Propodeum laterally usually with yellow spot (Fig. 6c; arrow). Clypeus breadth : malar space 3.85-4.55; head breadth : malar space 9.02-10.89; malar space : lateral ocelli distance $0.79-1.11$; metatibia length : malar space 9.57-11.5; terminal flagellomere length : malar space 1.00-1.24.

Males: Gena in dorsal view immediately narrowing behind eye (Fig. 12m). Epicnemium and mesosternum yellow. Head breadth : head height 1.21-1.29; lower
face : clypeus breadth 1.47-1.63; terminal flagellomere length : lateral ocelli distance 1.19-1.55; terminal flagellomere length : malar space 1.93-2.75; terminal flagellomere length : terminal flagellomere breadth 2.46-2.87.

Description of female. Body length $9.9-14.1 \mathrm{~mm}(\mathrm{n}=22)$; forewing length $7.8-$ 11.4 mm ( $\mathrm{n}=22$ ).

Head: Clypeus yellow, with a black margin and a large central black spot usually isolated (Fig. 6a) but seldom shaped like a (rhomboid) crossband reaching lateral margin. Face with large, almost triangular yellow spot touching inner orbit (Fig. 6a). Upper gena with small, elongate spot (Figs 6c, 6d). Frons with usually uninterrupted horizontal yellow stripe (Fig. 6a).

Mesosoma: Change in sculpture between coarse mesepisternum and smooth epicnemium frequently gradual (Fig. 12b). Pronotum along posterior margin with pair of longitudinal yellow stripes not reaching yellow cross stripe on pronotal collar (Fig. 6e). Scutellum with pair of yellow, somewhat triangular spots, followed by rectangular pair of spots on metanotum and crescent-shaped pair of spots on dorsal propodeum (Fig. 6e). Mesopleuron with yellow spot (Figs 6c, 6d). Propodeal valve yellow (Fig. 6c). Tegula yellow anteriorly and posteriorly, with transparent area in between (Fig. 6e). Legs apically yellow and orange, black only on coxa, trochanter and most of femur (Figs 6d, 6e), including base.

Metasoma: Each tergum with continuous, but indented terminal yellow band (Figs 6d, 6e). Tergum 2 also with two large yellow spots (Fig. 6e). Tergum 1 occasionally with two small yellow spots. Sterna 2 and 3 with continuous terminal yellow bands, on sternum 3 occasionally centrally indented close to interruption. Sternum 4 with interrupted terminal yellow band. Sternum 5 with broadly interrupted terminal band, reduced to two lateral yellow spots.

Description of male. Body length $11.3-13.4 \mathrm{~mm}(\mathrm{n}=8)$; forewing length 9.3$9.8 \mathrm{~mm}(\mathrm{n}=8)$.

Head: Mandibles, malar space, clypeus (Figs 7a, 7b), elongate spot on upper gena (Figs 7c, 7d, 7e), face and inferior frons yellow. Superior frons, vertex, occiput and back of head black (Figs 7a, 7c, 7e). Clypeus apically rounded (Fig. 7a), with faint lateral ridges extending toward orbital bays (Fig. 7b; arrow).

Mesosoma: Pronotum with yellow cross stripe along collar, often extending down both sides to longitudinal pair of yellow stripes along pronotal side margin (Fig. 7d; white arrow). Epicnemium and mesosternum yellow (Fig. 7d). Legs yellow and partially orange, except for superior side of coxa, trochanter and femur, which are black (Figs 7d, 7e). Rest of mesosoma colored as in females.

Metasoma: Tergum 2 with terminal yellow band laterally extending towards base (Fig. 7d; red arrow), even if occasionally interrupted. Terga otherwise colored as in females. Sternum 2 with pair of large yellow spots mostly isolated (Fig. 7d; black arrow), seldom fused. Sternum 3 with both terminal and basal yellow bands (Fig. 7d; blue arrow). Sterna 4 and 5 both with continuous terminal yellow band, the latter interrupted on sternum 6 and absent on hypopygium.

Comments. This is one of the smallest Polistes species in Europe and besides $P$. helveticus sp. n., the only one with often absent epicnemial carina in the female sex.

The two locally syntopic species (Fig. 11; Neumeyer et al. 2011) are, however, easy to distinguish in both sexes due to differently colored antennae. Furthermore, the ratio metatibia length : malar space is an unambiguous separator for females, whereas the best separating ratio for males ( $P$. bischoff, $P$. belveticus $\mathrm{sp} . \mathrm{n}$.) is the ratio terminal flagellomere length : malar space (Table 5). The same ratio weakly separates the sometimes similar females of $P$. bischoffi and $P$. gallicus. It is impossible to confuse the males of $P$. bischoffi with the males of $P$. hellenicus or $P$. biglumis due to the strikingly different color patterns and the diagnostic head shape of P. biglumis males within the gallicus-group.

Two morphs can be distinguished within $P$. bischoffi (rev. status), one with the flagellum entirely bright (yellow to orange) and the other with the flagellum dorsally faintly darkened. Often, the brighter morph (e.g. RN0137) has the clypeus with a central black spot (Fig. 6a), whereas in the darker morph the clypeus usually has a horizontal black band reaching the lateral margin. These two color morphs are probably the two extremes of an otherwise gradual continuum, but more individuals would have to be examined to verify this hypothesis. It would be even more important to examine whether such color variations are associated with geography or not. Limited evidence suggests that these variations are not associated with geographic location, since two nests were found (16 Aug 2013) in Zurich (Katzensee Allmend) with both morphs in each. In these colonies, the dark morph was more common among large females (presumably young queens), rather than among small females (presumably workers) or males. Also the neotype (RN0366) of bischoffi belongs to the darker morph and is presumably a queen, since it was collected on 19 April. More observations are needed to confirm this correlation between coloration and caste. Different color morphs within the same nest population are also reported in P. gallicus (Gusenleitner 1985: 105).

Distribution. Based on the material that we have examined, $P$. bischoffi occurs at least in Southern Europe and Turkey from the Atlantic coast of southern France to Turkish Kurdistan (Fig. 11). The northernmost confirmed locality is in the Pannonian region of Austria (Neusiedl am See), followed by several localities in Switzerland where the species occured already in 1927 at the river Versoix near Geneva (individuals RN0170, RN0171). In all other, more northern Swiss sites P. bischoffi occurs syntopically (Neumeyer et al. 2011) with $P$. helveticus sp. n. and was not detected before 1992, suggesting a possible recent range expansion due to climate warming.

Ecology. According to our experience in Switzerland, P. bischoffi appears to be restricted to large wetlands, especially to fens on lake shores, more so than $P$. helveticus sp. n . The altitudinal records range from sea level for several beach records (see "Material examined"), including the neotype (RN0366), to 540 m a.s.l. for a female (RN0076) in Switzerland (Wetzikon, Canton of Zurich). However, the Turkish locality (road from Yüksekova to Şemdinli) where three females (RN0363, RN0364, RN0365) were found was probably higher than 540 m a.s.l., since Yüksekova is situated at 1950 m , Şemdinli at 1450 m a.s.l., but the precise elevation of the locality is neither indicated on the label nor in the publication (Madl 1997: 824). Most individuals were found in August or September. The earliest record in the season is the neotype female from

Galeria on 19 Apr 2002, the latest a female from a still-active nest in Mönchaltorf on 10 September 2010. The earliest male (RN0022) recorded so far was captured at Pfäffikon (Switzerland) on 10 Aug 2011, whereas the latest males (RN0082, RN0083) recorded are from Wetzikon on 09 Sep 2011. Nesting habits are apparently similar to those of $P$. helveticus sp. n., even where the two species were encountered syntopically (Neumeyer et al. 2011). We also found two nests in Zurich (Katzensee Allmend, 16 Aug 2013) with more than 20 and 30 individuals, respectively. These two nests were larger than any of the 14 nests described by Neumeyer et al. (2011: 13). While the smaller of both nests was attached to the dry stem of an Apiaceae, the larger one was attached to the stem of a live yellow loosestrife (Lysimachia vulgaris).

Material examined. Neotype $q$ (RN0366): FRANCE, CORSICA, Galeria, $42^{\circ} 25^{\prime} 11^{\prime \prime} \mathrm{N}, 08^{\circ} 39^{\prime} 37^{\prime \prime} \mathrm{E}, 0 \mathrm{~m}, 19$ Apr 2002, estuary, Christian Monnerat leg., NMBE coll.

Paralectotype: $1 \nrightarrow$ (RN0287): GREECE, ATTICA, Poros, Moritz von Leonhardi (1856-1910) leg., SDEI coll., labeled as follows: 1. "Poros" [handwritten; misspelled as "Toros" in Weyrauch 1939: 164; see Blüthgen 1961: 56]; 2. "Coll. v. Leonhardi" [printed]"; 3. "Poliistes [sic!] \& gallicus L." [handwritten, possibly from v. Leonhardi; according to Stephan Blank (pers. comm.)]; 4. "Weyrauch det. 1937." [handwritten]; 5. "Polistula bischoff Weyrauch" [handwritten; possibly from Weyrauch; according to Stephan Blank (pers. comm.)]".

Further material: 1 q (RN0415): AUSTRIA, BURGENLAND, Neusiedl am See, 25 Jul 1989, Michael Madl leg., NHMW coll.; 1 \& (RN0414): 20 Aug 1991, Michael Madl leg., NHMW coll.; 1 q (RN0323): FRANCE, BOUCHES-DU-RHÔNE, Miramas, Étang de Berre, 15 Jul 1979, M. Kühbandner leg., MSNV coll.; 1 Q (RN0380): Saintes-Maries-de-la-Mer, Camargue, 28 Jul 2002, J. \& I. Smit leg., JS coll.; 1 q (RN0381): HÉRAULT, Vendres, $43^{\circ} 13^{\prime} 00^{\prime \prime} \mathrm{N}$, $03^{\circ} 1^{\prime} 4^{\prime} 38^{\prime \prime} \mathrm{E}, 0 \mathrm{~m}, 29$ Jul 2009, beach, J. \& I. Smit leg., JS coll.; 2 q (RN0382, RN0383): Palavas-les-Flots, 04 Jul 2005, dunes, J. \& I. Smit leg., JS coll.; 1 q (RN0379): LANDES, Vielle-Saint-Girons, Huchet, 04 Jul 2006, dunes, J. \& I. Smit leg., JS coll.; 2 Q (RN0367, RN0368): VAR, Fréjus, Saint-Aygulf, Jul 1924, Ferrière leg., NMBE coll.; 1 Q (RN0385): Roquebrune-sur-Argens, 14 Jul 2001, J. \& I. Smit leg., JS coll.; 1 \& (RN0384): road (D560) from Saint-Maximin-la-Sainte-Baume to Nans-les-Pins, 350 m, 15 Jul 2001, J. \& I. Smit leg., JS coll.; 1 o (RN0370): VAUCLUSE, Villelaure, 18 Jul 2000, Jan Smit leg., JS coll.; 1 q (RN0391): GREECE, ACHAEA, Kalogria, 01 Jul 2007, spit, Werner Arens leg., WA coll.; 1 q (RN0390): ARCADIA, Mantineia (archaeological site), 12 Jul 1997, Werner Arens leg., WA coll.; 1 q (RN0389): 06 Jul 2007, Werner Arens leg., WA coll.; 2 $q$ (RN0372, RN0373): EUBOEA, Chalkida, Camping Paradiso, 15 Jul 1982, M. \& G. Osella leg., MCSNV coll.; 2 \& (RN0392, RN0393): LACONIA, Chosiari, Vathi, 09 Jun 1998, beach, Werner Arens leg., WA coll.; 1 q (RN0322): ITALY, LAZIO, Roma, Torrimpietra, 10 Aug 1971, Heiss leg., MSNV coll.; 1 q (RN0410): Roma, Sep 1942, O. Querci, MSNM coll.; 1 Q (RN0411): LOMBARDIA, Guardamiglio, Fiume Po, 12 Aug 1974, river bank, Vincenzo Ferri
leg., MSNM coll.; 1 \& (RN0409): PIEMONTE, Cameri, Cascina Galdina, 11 Jul 1981, glade, Vincenzo Ferri leg., MSNM coll.; 1 ( ${ }^{(R N 0371): ~ L o m b a r d o r e, ~}$ Sep 1972, Osella leg., MCSNV coll.; 2 中 (RN0170, RN0171): SWITZERLAND, CANTON GENEVA, Versoix, "vers la Versoix", 1 Jul 1927, anon. leg., MHNG coll.; 1 q (RN0156): CANTON VAUD, Chabrey, La Morette, 8 Sep 1992, fen, Richard Vernier leg., MHNN coll.; 4 Q (RN0135, RN0136, RN0141, RN0148): CANTON ZURICH, Greifensee, Böschen: $47^{\circ} 22^{\prime} 21.46 " \mathrm{~N}, 08^{\circ} 40^{\prime} 03.38^{\prime \prime} \mathrm{E}, 436$ m, 11 Aug 2010, fen rotation fallow, Rainer Neumeyer leg., MZL coll.; $1 q+1 \delta^{\pi}$ (RN0147, RN0150): $47^{\circ} 22^{\prime} 21.46^{\prime \prime} \mathrm{N}, 08^{\circ} 40^{\prime} 03.38^{\prime \prime} \mathrm{E}, 436 \mathrm{~m}, 11$ Aug 2010, fen rotation fallow, Rainer Neumeyer leg., AMNH coll.; 1 q (RN0146): $47^{\circ} 22^{\prime} 20.36^{\prime \prime} \mathrm{N}$, $08^{\circ} 40^{\prime} 02.49^{\prime \prime} \mathrm{E}, 436 \mathrm{~m}, 11$ Aug 2010, fen rotation fallow, Rainer Neumeyer leg., CM coll.; 1 中 (RN0142): $47^{\circ} 22^{\prime} 20.59^{\prime \prime} \mathrm{N}, 08^{\circ} 40^{\prime} 02.64$ "E, $436 \mathrm{~m}, 11$ Aug 2010, fen rotation fallow, Rainer Neumeyer leg., MH coll.; 1 q (RN0137) + 1 § (RN0151): Mönchaltorf, Seewisen, $47^{\circ} 19^{\prime} 17.08^{\prime \prime N}, 08^{\circ} 41^{\prime} 56.05^{\prime \prime} \mathrm{E}, 436 \mathrm{~m}, 11$ Aug 2010, fen rotation fallow, Rainer Neumeyer leg., NMBE coll.; 1 q (RN0140): $47^{\circ} 19^{\prime} 17.80^{\prime \prime} \mathrm{N}$, $08^{\circ} 41^{\prime} 54.97^{\prime \prime} \mathrm{E}, 436 \mathrm{~m}, 11$ Aug 2010, fen rotation fallow, Rainer Neumeyer leg., CSE coll.; 1 Q (RN0001): Pfäffikon, Auslikon, $47^{\circ} 20^{\prime} 47.7^{\prime \prime} \mathrm{N}, 08^{\circ} 47^{\prime} 50.16^{\prime \prime} \mathrm{E}, 539$ m, 10 May 2011, fen, Rainer Neumeyer leg., RN coll.; 1 § (RN0022): Pfäffikon, Birchen, $47^{\circ} 21^{\prime} 03.19^{\prime \prime} \mathrm{N}, 08^{\circ} 47^{\prime} 31.73^{\prime \prime} \mathrm{E}, 538 \mathrm{~m}, 10$ Aug 2011, fen, Rainer Neumeyer leg., RN coll.; 1 \& (RN0169): Regensdorf, Altburg, 24 Jul 1997, hill near fen, Bernhard Merz leg., MHNG coll.; 1 q (RN0145): Schwerzenbach, Böschen, $47^{\circ} 22^{\prime} 21.48^{\prime \prime} \mathrm{N}, 08^{\circ} 40^{\prime} 01.19^{\prime \prime} \mathrm{E}, 436 \mathrm{~m}, 11$ Aug 2010, fen rotation fallow, Rainer Neumeyer leg., NML coll.; 1 q (RN0149): $47^{\circ} 22^{\prime} 21.35^{\prime \prime} \mathrm{N}, 08^{\circ} 40^{\prime} 00.85^{\prime \prime} \mathrm{E}, 436 \mathrm{~m}$, 11 Aug 2010, fen rotation fallow, Rainer Neumeyer leg., NHMB coll.; 1 q (RN0143): $47^{\circ} 22^{\prime} 21.39^{\prime \prime} \mathrm{N}, 08^{\circ} 40^{\prime} 00.75^{\prime \prime} \mathrm{E}, 436 \mathrm{~m}, 11$ Aug 2010, fen rotation fallow, Rainer Neumeyer leg., ETHZ coll.; 1 q (RN0144): $47^{\circ} 22^{\prime} 21.39^{\prime \prime} \mathrm{N}, 08^{\circ} 40^{\prime} 00.90$ "E, 436 m, 11 Aug 2010, fen rotation fallow, Rainer Neumeyer leg., MCSNL coll.; 1 q (RN0076): Wetzikon, Himmerich: $47^{\circ} 20^{\prime} 07.5^{\prime \prime} \mathrm{N}, 08^{\circ} 47^{\prime} 31.05^{\prime \prime} \mathrm{E}, 540 \mathrm{~m}, 02$ Sep 2011, fen, Rainer Neumeyer leg., RN coll.; 1 \& (RN0105): Wetzikon, Robenhuserriet, $47^{\circ} 20^{\prime} 11.31^{\prime \prime} \mathrm{N}, 08^{\circ} 47^{\prime} 14.84^{\prime \prime} \mathrm{E}, 538 \mathrm{~m}, 17$ Aug 2012, fen, Rainer Neumeyer
 538 m, 02 Sep 2011, fen, Rainer Neumeyer leg., RN coll.; 2 § (RN0082, RN0083): $47^{\circ} 20^{\prime} 30.24^{\prime \prime} \mathrm{N}, 08^{\circ} 47^{\prime} 10.89^{\prime \prime} \mathrm{E}, 538 \mathrm{~m}, 09$ Sep 2011, fen, Rainer Neumeyer leg., RN coll.; 1 q (RN0077): $47^{\circ} 20^{\prime} 29.54^{\prime \prime} \mathrm{N}, 08^{\circ} 47^{\prime} 23.73^{\prime \prime} \mathrm{E}, 537 \mathrm{~m}, 02$ Sep 2011, fen, Rainer Neumeyer leg., RN coll.; 1 § (RN0328): Zürich, Katzensee Allmend, $47^{\circ} 25^{\prime} 53.05^{\prime \prime} \mathrm{N}, 08^{\circ} 30^{\prime} 26.5^{\prime \prime} \mathrm{E}, 438 \mathrm{~m}, 16$ Aug 2013, fen, André Rey leg., AR coll.; 4 ¢ (RN0329, RN0330, RN0331, RN0332) + 3 § (RN0333, RN0335, RN0336): 19 Aug 2013, Rainer Neumeyer leg., RN coll.; 1 ő (RN0334): 19 Aug 2013, Rainer Neumeyer leg., NHM coll.; 2 ¢ (RN0337, RN0338) + 2 § (RN0339, RN0340): $47^{\circ} 25^{\prime} 56.74^{\prime \prime} \mathrm{N}, 08^{\circ} 30^{\prime} 22.41^{\prime \prime} \mathrm{E}, 438 \mathrm{~m}, 19$ Aug 2013, fen, Rainer Neumeyer leg., RN coll.; 3 \& (RN0363, RN0364, RN0365): TURKEY, HEKARÎ, road from Yüksekova to Şemdinli ["Sendili"], 1450-1950 m, marshy meadow [Sumpfwiese], 05 Jun 1971, Michael Madl leg., NHMW coll.

## Polistes gallicus (Linnaeus, 1767)

http://species-id.net/wiki/Polistes_gallicus
Vespa gallica Linnaeus, 1767: 949 - Holotype male (LSL), type locality "Europa australi" [South of Europe] (not examined)
Polistes foederatus Kohl, 1898: 90 - Lectotype male (NHMW), designated by Blüthgen (1943: 129), type locality Göygöl ["Transkauk., Helenendorf"], Azerbaijan (examined by RN)
Polistes gallicus mongolicus Buysson, 1911: 218 - Syntypes males females (MNHN, ZIN), type locality road from Kuqa ["Koutchar"] to Karashahr ["Karachar"], China (Xinjiang autonomous region) (syntype male in MHNG examined by RN)
Polistes foederatus obscuricornis Mader, 1936: 263 - Syntypes females (NHMW), type locality (island of) Krk, Croatia ( 2 syntypes examined by RN)
Polistula omissa Weyrauch, 1938: 277 - Lectotype male (lost; see Arens 2011: 462), designated by Weyrauch 1939: 161, type locality Marseille, France, mentioned in Weyrauch (1939): 161.
Polistes omissus ordubadensis Zirngiebl, 1955: 381 - Holotype female (ZSM), type locality Ordubad, Azerbaijan (examined by RN)
Polistes omissus kaszabi Giordani Soika, 1970: 327-328 - Holotype female (HNHM), type locality "Duusch ul" near Züünkharaa [,Zuun-Chara"], Mongolia (examined by RN) Polistes foederatus albellus Giordani Soika, 1976: 272 - Holotype female (HNHM, currently loaned elsewhere), type locality Bulgan aimag: Namnan ul mountains, 23 km NW of Somon Chutag, Mongolia (1 paratype female in MSNV examined by RN)

Diagnosis. Relatively small and bright species with flagellum bright yellow to orange on upper side in both sexes; pedicel and extreme base of flagellomere 1 always black on upper side.

Females: Epicnemial carina distinct or reduced. Hypopygium black. Metacoxa frequently spotted yellow on upper side. Mesoscutum often with pair of yellow spots. Propodeum laterally with yellow spot on each side. Clypeus breadth : malar space 3.49-4.25; head breadth : malar space 8.23-10.08; malar space : lateral ocelli distance 0.87-1.2; metatibia length : malar space 8.26-11.88; terminal flagellomere length : malar space $0.84-1.07$.

Males: Gena in dorsal view immediately narrowing behind eye. Epicnemium and mesosternum yellow. Head breadth : head height 1.17-1.27; lower face : clypeus breadth 1.46-1.6; terminal flagellomere length : lateral ocelli distance 1.09-1.55; terminal flagellomere length : malar space 1.28-2.21; terminal flagellomere length : terminal flagellomer breadth 1.84-3.2.

Comments. The holotype of Vespa gallica Linnaeus, 1767 (LINN 2790), presently held at the Linnean Society of London, is not available for loan. We have, however, examined pictures (http://linnean-online.org/16757/). They clearly show the bright flagellum all around, excluding identity with $P$. helveticus sp. n. A careful examination of this specimen would be needed to confirm the identify of $P$. gallicus.

Except for Vespa gallica and for the lost lectotype of Polistula omissa Weyrauch, 1938 we have examined and measured type specimens of all taxa listed as synonymous with $P$. gallicus. Since each of them appears to be clearly distinct from $P$. bischoffi in our morphometric analyses (Fig. 3), we only compare them with $P$. helveticus sp. n. in the following section.

Blüthgen (1941: 245) and Guiglia (1972: 49) claim that the upper side of the flagellum of P. foederatus Kohl, 1898 is "slightly blackened [leicht geschwärzt]" or "darkened [assombrie]", respectively, even in the male sex, unlike the flagellum of P. omissus (Weyrauch, 1938). However, the flagellum of the male lectotype of $P$. foederatus from Azerbaijan is bright yellow all around, as noted by Blüthgen (1943: 129), thus excluding any confusion with $P$. helveticus sp. n. Furthermore, the clypeus of this lectotype has not even a trace of a longitudinal furrow, although this trait has also been regarded as diagnostic for $P$. foederatus (Blüthgen 1941: 245). Currently, both $P$. foederatus and P. omissus are synonyms of gallicus (Day 1979: 63; Gusenleitner 1985: 105). This view is supported by our morphometric analysis (Fig. 3b, d [J]) for P. foederatus.

The lectotype of Polistes gallicus mongolicus Buysson, 1911 has its epicnemium and mesosternum largely black as in $P$. hellenicus, but is otherwise a large, very light colored male with an extremely broad head. Its flagellum is bright all around, excluding any synonymy with $P$. helveticus sp . n . The terminal flagellomere is in fact "very short" [très court], as Buysson (1911: 218) states. Morphologically it appears doubtful that the taxon mongolicus belongs to $P$. gallicus (C. van Achterberg, pers. comm.). This view is also supported by our morphometric analyses (Fig. 3b, d [K]).

We have seen two syntypes (RN0444, RN0445) of Polistes foederatus obscuricornis Mader, 1936. In contrast to the statement of Mader (1936: 263) the flagella of these two females are not "entirely black [ganz schwarz]" dorsally, but only grey. Only the scape, the pedicel, and the very base of the first flagellomere are entirely black dorsally. Both of these otherwise light colored individuals have a pronounced epicnemial carina, excluding confusion with P. helveticus sp. n. Although this taxon belongs without any doubt to the gallicus-group, our morphometric analyses (Fig. 3a, c [D]) do not entirely support their synonymy with $P$. gallicus.

The female holotype of Polistes omissus ordubadensis Zirngiebl, 1955 is colored very light with the clypeus unspotted, the flagellum colored light dorsally, the hypopygium (Blüthgen 1956: 85) and even the malar space largely yellow, and the epicnemial carina distinct, all of them excluding confusion with $P$. helveticus sp . n . This view is also supported by morphometry (Fig. 3a, c [E]). In our opinion this taxon (P. omissus ordubadensis) may not even belong to the gallicus-group.

The holotype of Polistes omissus kaszabi Giordani Soika, 1970 is a large, dark female with both malar space and mandibles entirely black. The epicnemial carina is distinct, excluding confusion with $P$. helveticus sp. n. Flagellum, clypeus and hypopygium are colored and patterned as in $P$. biglumis or $P$. nimpha. In fact, a synonymy with $P$. gallicus is not supported by our morphometric analyses (Fig. 3a, c [F]) and this taxon (P. omissus kaszabi) may not even belong to the gallicus-group (C. van Achterberg, pers. comm.).

The holotype of Polistes foederatus albellus Giordani Soika, 1976 is currently on loan and could not be examined, but we have examined the only paratype (RN0326, MSNV-04702) mentioned in Giordani Soika (1976: 272). It is an extremely dark female with the flagellum dorsally black and the epicnemial carina unilaterally reduced. It is morphologically and even morphometrically (Fig. 3a, c [G]) similar to a very dark $P$. helveticus sp. n., except that the bright spots and stripes are not only reduced, but also of ivory-white rather than of yellow color. These striking characters make it unlikely that this taxon (albellus) belongs to a species reaching Europe (C. van Achterberg, pers. comm.).

These observations indicate that the taxon gallicus should be carefully revised; our analysis of the mitochondrial marker further indicates that two specimens ( $P$. sp. aff. gallicus; RN0126, RN0129) from Greece, both identified as $P$. gallicus by Arens (2011), may represent another species. The upper side of the flagellum in both of these individuals is slightly darkened. This trait, as well as others (e.g. a broad malar space), suggest that this taxon ( $P$. sp. aff. gallicus) may be P. foederatus obscuricornis. More data are required to resolve this problem.

In addition, our morphometric analysis reveals considerable intraspecific heterogeneity within P. gallicus. Specimens from continental Europe (Croatia, Portugal, Switzerland) were quite different from those from Sardinia. Both taxa overlapped but exhibited considerable differences. However, our molecular analyses did not indicate any difference between continental and Sardinian specimens. Therefore, we must also consider some unknown external factors such as temperature or humidity to be at least partially responsible for the observed morphometric variation. Such factors have previously been implicated in color pattern variation (Zimmermann 1931).

Material examined. $1 \delta$ (Lectotype of $P$. foederatus): AZERBAIJAN, GOYGOL DISTRICT, Göygöl ["Transkauk., Helenendorf"], 1886, Franz Friedrich Kohl det., NHMW coll.; 1 q (Holotype of P. omissus ordubadensis: ZSM-HYM-000005): NAKHCHIVAN AUTONOMOUS REPUBLIC, Ordubad, 1913, Kulzer leg., ZSM coll.; 1 § (Lectotype of P. gallicus mongolicus; EY8898): CHINA, XINJIANG, road from Kuqa ["Koutchar"] to Karashahr ["Karachar"] in Kashgar prefecture ["Kachgarie"], 1909, L. Vaillant leg., MNHN coll.; 1 \& (RN0099): CROATIA, ISTRIA, Rovinj, Cesta za Mondelaco, $45^{\circ} 05^{\prime} 57.6^{\prime \prime} \mathrm{N}, 13^{\circ} 38^{\prime} 36.8^{\prime \prime} \mathrm{E}, 15 \mathrm{~m}, 24 \mathrm{Jul} 2012$, fallow, Rainer Neumeyer leg., CSE coll.; 1 q (RN0100): 28 Jul 2012, fallow, Rainer Neumeyer leg., CSE coll.; 3 Q (RN0194, RN0195, RN0196): Rainer Neumeyer leg., RN coll.; 1 Q (RN0009): Rovinj, Farma Haber, $45^{\circ} 06^{\prime} 40.8^{\prime \prime} \mathrm{N}, 13^{\circ} 40$ '21.0"E, 30 m, 22 Jul 2011, farm, Rainer Neumeyer leg., RN coll.; 1 ¢ (RN0197): Vodnjan, street D21, $44^{\circ} 57^{\prime} 58.0^{\prime \prime} \mathrm{N}, 13^{\circ} 50^{\prime} 47.4^{\prime \prime} \mathrm{E}, 133 \mathrm{~m}, 24 \mathrm{Jul} 2012$, fallow, Rainer Neumeyer leg., RN coll.; 2 q (Syntypes of P. foederatus obscuricornis; RN0444, RN0445): KRK, $\leq 1936$, NHMW coll.; 1 Q (RN0343): ITALY, SARDINIA, Cabras, Stagno di Cabras, $39^{\circ} 57^{\prime} 07.6^{\prime \prime} \mathrm{N}, 08^{\circ} 31^{\prime} 17.2^{\prime \prime} \mathrm{E}, 5 \mathrm{~m}, 31$ Aug 2013, paddy, Rainer Neumeyer leg., RN coll.; $1 \widehat{o}^{\lambda}$ (RN0347): Strada Provinciale 1, $39^{\circ} 55^{\prime} 14.1^{\prime \prime} \mathrm{N}, 08^{\circ} 31^{\prime} 17.4^{\prime \prime} \mathrm{E}, 6$ m, 01 Sep 2013, reed, Rainer Neumeyer leg., RN coll.; 1 \& (RN0344): Macomer, Via Alagon, $40^{\circ} 15^{\prime} 49.9^{\prime \prime} \mathrm{N}, 08^{\circ} 46^{\prime} 54.5^{\prime \prime} \mathrm{E}, 553 \mathrm{~m}, 01$ Sep 2013, rock face, Rainer Neu-
meyer leg., RN coll.; 1 Q (RN0345) + $1 \circlearrowleft$ (RN0348): Scano di Montiferro, Street SP78, $40^{\circ} 13^{\prime} 19.9^{\prime \prime} \mathrm{N}, 08^{\circ} 36^{\prime} 29.9^{\prime \prime} \mathrm{E}, 442 \mathrm{~m}, 01$ Sep 2013, quarry, Rainer Neumeyer leg., NHM coll.; $1 \sigma^{\top}$ (RN0346): Tadasuni, Street to San Serafino, $40^{\circ} 05^{\prime} 41.4^{\prime \prime} \mathrm{N}$, $08^{\circ} 52^{\prime} 44.5^{\prime \prime} \mathrm{E}, 138 \mathrm{~m}, 30$ Aug 2013, edge community, Rainer Neumeyer leg., RN coll.; 1 Q (Paratype of $P$. foederatus albellus; RN0326, MSNV-04702): MONGOLIA, BULGAN AIMAG, Namnan ul mountains, 1150 m, 21 Jul 1968, Zoltán Kaszab leg., Antonio Giordani Soika det., MSNV coll.; 1 \& (Holotype of P. omissus kaszabi; HNHM-283): SELENGE AIMAG, "Duusch ul" near Züünkharaa [„Zuun-Chara"], 1100 m, 08 Jul 1964, Zoltán Kaszab leg., HNHM coll.; 1 q (RN0108): PORTUGAL, ALGARVE, Vila do Bispo, western shore, $37^{\circ} 06^{\prime} 57.2^{\prime \prime} \mathrm{N}, 08^{\circ} 55^{\prime} 37.2^{\prime \prime} \mathrm{W}$, 11 m, 04 Apr 2012, dune, Rainer Neumeyer leg., RN coll.; 1 \& (RN0395) + $1 \delta^{\pi}$ (RN0396): SLOVAKIA, NITRA REGION, Štúrovo, 16 Sep 1947, Augustin Hoffer leg., LD coll.; 1 § (RN0172): SWITZERLAND, CANTON GENEVA, Genève, < 1900, anon. leg., MHNG coll.; 1 § (RN0208): CANTON GRISONS, Roveredo, 18 Aug 1924, Adolf Nadig leg., ETHZ coll.; 3 § (RN0205, RN0206, RN0207): 17 Aug 1949, Adolf Nadig leg., ETHZ coll.; 1 q (RN0118): San Vittore, Ai Tecc, $46^{\circ} 14^{\prime} 10.42^{\prime \prime} \mathrm{N}, 09^{\circ} 05^{\prime} 20.18^{\prime \prime} \mathrm{E}, 262 \mathrm{~m}, 30 \mathrm{Jul} 2012$, industrial fallow, Rainer Neumeyer leg., RN coll.; $1 \delta$ (RN0166): Savognin, Jul 1907, anon. leg., BNM coll.; 1 q (RN0165): Val Müstair, Sta. Maria, 20 Jul 1951, Jacques de Beaumont leg., BNM coll.; 10 § (RN0173, RN0209, RN0210, RN0211, RN0212, RN0213, RN0214, RN0215, RN0216, RN0217): CANTON TICINO, Locarno, 05 Jul 1907, anon. leg., ETHZ coll.; 3 q (RN0110, RN0111, RN0112): Sant' Antonino, Via Gorelle/Canvera, $46^{\circ} 09^{\prime} 19.72^{\prime \prime} \mathrm{N}, 08^{\circ} 58^{\prime} 18.65^{\prime \prime} \mathrm{E}, 215 \mathrm{~m}, 30 \mathrm{Jul} 2012$, industrial fallow, Rainer Neumeyer leg., RN coll.; 4 \& (RN0103, RN0106, RN0107, RN0109): 08 Aug 2012, industrial fallow, Rainer Neumeyer leg., RN coll.; 2 (RN0041, RN0042): CANTON VALAIS, Leuk, Satellitenbodenstation, $46^{\circ} 19^{\prime} 06.66^{\prime \prime} \mathrm{N}, 07^{\circ} 38^{\prime} 36.56^{\prime \prime} \mathrm{E}$, 919 m, 22 Aug 2011, tall herbaceous vegetation, Rainer Neumeyer leg., RN coll.; 2 ㅇ (RN0104, RN0113): 07 Aug 2012, Rainer Neumeyer leg., RN coll.; 1 + +2 ठ (RN0115, RN0116, RN0117): CANTON VAUD, Villars-sous-Yens, Arborex, $46^{\circ} 30^{\prime} 11.03^{\prime \prime} \mathrm{N}, 06^{\circ} 25^{\prime} 12.11$ " $\mathrm{E}, 510 \mathrm{~m}, 25$ Aug 2010, Christian Monnerat leg., MHNN coll.

## Polistes hellenicus Arens, 2011

http://species-id.net/wiki/Polistes_hellenicus
Polistes hellenicus Arens, 2011: 464 - Holotype male (WA), type locality Ano Kotili, Greece (examined by RN)

Diagnosis. Relatively small, bright species with flagellum bright yellow on upper side in both sexes; pedicel and extreme base of flagellomere 1 always black on upper side.

Females: Epicnemial carina distinct. Hypopygium often orange and yellow at the tip. Metacoxa spotted yellow on upper side. Mesoscutum sometimes with pair of yel-
low spots. Propodeum laterally often with yellow spot on each side. Clypeus breadth : malar space 3.46-4.46; head breadth : malar space 7.75-10.65; malar space : lateral ocelli distance $0.85-1.24$; metatibia length : malar space 9.00-11.13; terminal flagellomere length : malar space $0.74-1.11$.

Males: Gena in dorsal view immediately narrowing behind eye. Epicnemium and mesosternum black (Fig. 8). Head breadth : head height 1.15-1.21; lower face : clypeus breadth 1.48-1.62; terminal flagellomere length : lateral ocelli distance 1.21-1.4; terminal flagellomere length : malar space 1.44-2.16; terminal flagellomere length : terminal flagellomere breadth 2.12-2.76.

Comments. The male holotype of $P$. hellenicus can be easily distinguished from P. bischoffi and $P$. helveticus sp. n. based on its black epicnemium and mesosternum (Fig. 8). From a morphometric point of view the holotype (hellenicus) lies at the border with $P$. bischoff , but clearly outside the range of $P$. helveticus sp. n. (Fig. 3b, c [L]).

Material examined. Holotype $\begin{gathered} \\ \text { (RN0242): GREECE, ARCADIA, Ano Kotili, }\end{gathered}$ peak of Lykaion, 1100-1400 m, 07 Jul 2010, Werner Arens leg., WA coll.

Paratypes: 1 甲 (RN0174): GREECE, ARCADIA, Andritsena, 1997, Werner Arens leg., ZSM coll.; $1 \circlearrowleft^{\top}$ (RN0125): Ano Kotili, peak of Lykaion, 1100-1400 m, 07 Jul 2010, Werner Arens leg., WA coll.; 1 § (RN0179): ARGOLIS, Drepano, 06 Jul 2008, Werner Arens leg., WA coll.

Further material: 4 § (RN0220, RN0221, RN0222, RN0223): CROATIA, ISTRIA, Ližnjan, $44^{\circ} 49^{\prime}$ N, $13^{\circ} 59^{\prime} E, 27$ Aug 2005, Christian Schmid-Egger leg., CSE coll.; 2 q (RN0008, RN0088): Rovinj, $45^{\circ} 06^{\prime} 07.1^{\prime \prime N}$, $13^{\circ} 39^{\prime} 08.4^{\prime \prime} \mathrm{E}, 22 \mathrm{~m}, 22 \mathrm{Jul}$ 2011, Rainer Neumeyer leg., RN coll.; 1 (RN0097): Rovinj, Cesta za Mondelaco, $45^{\circ} 05^{\prime} 57.6^{\prime \prime} \mathrm{N}, 13^{\circ} 38^{\prime} 36.8^{\prime \prime} \mathrm{E}, 15 \mathrm{~m}, 24 \mathrm{Jul} 2012$, Rainer Neumeyer leg., CSE coll.; 1 ¢ (RN0098): 28 Jul 2012, Rainer Neumeyer leg., CSE coll.; 1 q (RN0193): Rainer Neumeyer leg., RN coll.; 2 ( $\uparrow$ (R0010, RN0011): Rovinj, Cesta za Valaltu-Lim, $45^{\circ} 06^{\prime} 16.8^{\prime \prime} \mathrm{N}, 13^{\circ} 38^{\prime} 28.4^{\prime \prime} \mathrm{E}, 26 \mathrm{~m}, 26$ Jul 2011, Rainer Neumeyer leg., RN coll.; 1 $\uparrow$ (RN0191): 21 Jul 2012, Rainer Neumeyer leg., RN coll.; 1 q (RN0193): Rovinj, near Valalta, $45^{\circ} 07^{\prime} 02.1^{\prime \prime} \mathrm{N}, 13^{\circ} 37^{\prime} 54.0^{\prime \prime} \mathrm{E}, 14 \mathrm{~m}, 21 \mathrm{Jul} 2012$, Rainer Neumeyer leg., RN coll.; $2 \oint^{\top}$ (RN0101, RN0102): Vela Učka, $45^{\circ} 18^{\prime} 25.7^{\prime \prime} \mathrm{N}, 14^{\circ} 11^{\prime} 40.4^{\prime \prime} \mathrm{E}, 824 \mathrm{~m}$, 27 Jul 2012, karst mountain range, Rainer Neumeyer leg., RN coll.; 1 Q (RN0183): GREECE, ARCADIA, Andritsena, 31 May 2011, Werner Arens leg., WA coll.; 1 ㅇ (RN0132): Mantinea, 08 Jun 2011, Werner Arens leg., WA coll.; 1 Q (RN0181): ELIS, Olympia, 29 May 2011, Werner Arens leg., WA coll.; 1 Q (RN0131): 08 Jun 2011, Werner Arens leg., WA coll.; 1 Q (RN0182): Zacharo, Lake Kaiapha, 30 May 2011, Werner Arens leg., WA coll.; 1 q (RN0096): Zacharo, Neochori, 30 May 2011, Werner Arens leg., RN coll.; 1 q (RN0175): IONIAN ISLANDS, Cephalonia, 03 Sep 1992, Peter Hartmann leg., ZSM coll.; 1 q (RN0127): MESSENIA, Avia near Kalamata, 01 Jun 2011, Werner Arens leg., WA coll.; 3 \& (RN0133, RN0185, RN0186): 02 Jun 2011, Werner Arens leg., WA coll.; 1 \& (RN0176): Kalamata, 1997, Werner Arens leg., ZSM coll.; 1 Q (RN0184): Mavromati, Ithome mountain, 01 Jun 2011, Werner Arens leg., WA coll.; 1 ( q (R0177): 1997, Werner Arens leg., ZSM coll.


Figure 8. Lateral aspect of the holotype (individual RN0242; male) of Polistes hellenicus Arens, 2011. Unique for males of European Polistes is the black ventral side of the mesosoma, especially epicnemium and mesosternum.

## Polistes helveticus Neumeyer, sp. n.

http://zoobank.org/2BF81BF0-5EB0-4C74-87C4-50A294C330A3
http://species-id.net/wiki/Polistes_helveticus
Polistes bischoffi Weyrauch, 1937: 274, in part - Weyrauch (1939), in part (paralectotype male, RN0325, of P. bischoffi, HUMCZ coll., Glattbrugg near Zurich, Switzerland). The following references published under the name of Polistes bischoffi Weyrauch, 1937 actually belong to P. helveticus sp. n.: Baugnée (1996), Blüthgen (1961), Dvořák and Roberts (2006), Dvořák et al. (2006), Graf (1961), Guiglia (1967, 1972), Kofler (2005), Mauss (2001), Mauss and Treiber (2004), Neumeyer et al. (2011), Schmid-Egger and Treiber (1989), Schneider et al. (1998), Witt (2009).

Holotype female (NMBE coll.), present designation, type locality Schwerzenbach, canton of Zürich, Switzerland

Diagnosis. Small and relatively dark species with pedicel and flagellum black on upper side in both sexes (Figs 9a, 9b, 10a, 10c).

Females: Epicnemial carina reduced (Fig. 9c; red arrow) or absent. Hypopygium black (Fig. 9d). Metacoxa black. Mesoscutum black (Fig. 9e), only rarely with a minute pair of yellow spots. Propodeum laterally usually black (Fig. 9d), seldom with yellow spot on each side. Clypeus breadth : malar space 3.26-3.73; head breadth : malar space 7.29-8.8; malar space : lateral ocelli distance 0.87-1.19; metatibia length : malar space 7.61-9.33. terminal flagellomere length : malar space 0.81-1.07.

Males: Gena in dorsal view immediately narrowing behind eye (Fig. 12m). Epicnemium and mesosternum yellow (Figs 10a, 10c). Head breadth : head height 1.191.27; lower face : clypeus breadth 1.46-1.69; terminal flagellomere length : lateral ocelli distance $0.83-1.32$; terminal flagellomere length : malar space $1.08-1.72$; terminal flagellomere length : terminal flagellomere breadth 1.70-2.78.


Figure 9. Different aspects of a female (individual RN0138) of Polistes helveticus sp. n.: a frontal view of head $\mathbf{b}$ lateral view of lower face with malar space and mandible $\mathbf{c}$ mesopleural region of mesosoma d lateral view of body $\mathbf{e}$ dorsal view of body. The red arrow in picture (c) is pointing to the rather reduced epicnemial carina, and the white arrow to the quite distinct mesopleural signum (sensu Carpenter 1996a), a structure also called a sternopleural groove (Richards 1973).

Description of female. Body length $9.7-14.1 \mathrm{~mm}(\mathrm{n}=20)$; forewing length $8.5-$ $11.3 \mathrm{~mm}(\mathrm{n}=20)$.

Head: Clypeus yellow with black margin and large central black spot; this spot either isolated (Fig. 9a) or more often extended as crossband reaching the lateral margins of clypeus (Fig. 12g). Face with nearly triangular yellow spot touching inner orbit (Fig. 9a). Upper gena with small, elongate spot (Fig. 9d). Frons with pair of horizontal yellow stripes seldom confluent (Fig. 9a).

Mesosoma: Change in sculpture between coarse mesepisternum and smooth epicnemium frequently gradual (Fig. 12b). Pronotum along posterior margin with pair of longitudinal stripes not reaching cross stripe on pronotal collar (Fig. 9e). Scutellum and metanotum each with pair of yellow bars (Fig. 9e). Propodeum dorsally usually


Figure IO. Different aspects of a male (individual RN0153) of Polistes helveticus sp. n.: a frontal view of head $\mathbf{b}$ anterolateral view of lower face $\mathbf{c}$ lateral view of body $\mathbf{d}$ dorsal view of body. The arrows are pointing to the isolated black area (a) bordering the torulus, the very faint lateral ridges of clypeus (b) or the ventrolateral angle of the pronotum (c).
with pair of crescent-shaped spots (Fig. 9e). Mesopleuron with yellow spot (Figs 9c, 9d). Propodeal valve yellow (Fig. 9d). Tegula yellow anteriorly and posteriorly, with more transparent area in between (Fig. 9e). Legs yellow and orange, black only on coxa, trochanter and most of femur, including entire base (Figs 9d, 9e).

Metasoma: Each tergum with continuous, but slightly indented terminal yellow band (Figs 9d, 9e). Tergum 2 also with two yellow spots (Fig. 9e). Tergum 1 seldom with two small yellow spots. Sterna 2 and 3 with terminal yellow bands usually inter-
rupted, even though often only slightly so. Sterna 3, 4 and 5 with broadly interrupted terminal bands, manifested only as lateral terminal yellow spots.

Description of male. Body length $9.6-12.4 \mathrm{~mm}(\mathrm{n}=12)$; forewing length 8.9$9.9 \mathrm{~mm}(\mathrm{n}=12)$.

Head: Mandibles, malar space, clypeus, face, inferior frons (Figs 10a, 10b) and elongate spot on upper gena (Fig. 10c) yellow. Superior frons, vertex (Fig. 10a), occiput and back of head (Figs 10c, 10d) black. Inferior part of frons yellow with small black area at superior margin of torulus, usually isolated (Fig. 10a; arrow), but seldom reaching the superior part of frons above. Clypeus apically rounded (Fig. 10a), with hardly any lateral ridge (Fig. 10b; arrows).

Mesosoma: Pronotum with yellow cross stripe along collar, occasionally extending down to sharp angle of pronotum (Fig. 10c; arrow). Legs yellow and orange, except for upper sides of coxa, trochanter and femur, which are black (Figs 10c, 10d); black area occasionally reaching (yellow) lower side of hind femur, yellow area occasionally reaching (black) upper side of pro- and mesocoxa. Rest of mesosoma colored as in females (Fig. 10d).

Metasoma: Tergum 2 with terminal yellow band extending laterally toward base, even if occasionally discontinuous. Other terga colored as in females. Sternum 2 most of the time with two yellow spots. Sterna 3, 4 and 5 usually with continuous terminal yellow band, the latter interrupted on sternum 6 and absent on hypopygium.

Comments. Except for P. bischoffi, Polistes helveticus sp. n. is the only European species with an epicnemial carina that is often absent in the female sex. These two species are easy to distinguish in both sexes due to their differing color patterns, mainly on the antennae. Furthermore, the ratio metatibia length : malar space separates females, whereas the best separating ratio for males ( $P$. bischoff, $P$. helveticus sp. n .) is terminal flagellomere length : malar space (Table 5). Confusion with P. gallicus or P. hellenicus is unlikely due to the very different color patterns in both sexes. Males are virtually impossible to confuse with P. biglumis; however, the very similarly colored females of $P$. helveticus sp. n. and $P$. biglumis are likely to be confused in specimens of $P$. helveticus sp. n. with an exceptionally developed epicnemial carina. For such cases, we provide the ratio malar space : lateral ocelli distance, which fully separates the two.

Since most collected specimens labeled as "Polistes bischoffi" are presumed to belong to P. helveticus sp. n., rather than to P. bischoffi Weyrauch, 1937 (revised status), at least in Central European museums, their identity must be checked. In fact, according to the CSCF (www.cscf.ch; in litt.) there are about 450 individuals of $P$. helveticus sp. n. from Switzerland deposited in Swiss museums, but only very few ( $<10$ ) individuals of $P$. bischoffi, at least before the material of the present study was deposited. A similar situation may apply to other Central European museums, especially in Austria and Germany. In contrast, the relatively few individuals labeled as " $P$. bischoff" that we examined from Southern Europe (Greece, Italy, Southern France) are, in fact, determined correctly (mostly by Josef Gusenleitner).

The specimen that we have chosen as the holotype of $P$. helveticus $\mathrm{sp} . \mathrm{n}$. clearly belongs to $P$. helveticus sp. n. according to molecular and morphological analyses. Ac-


Figure I I. Distribution of examined specimens of Polistes bischoff Weyrauch, 1937 and Polistes helveticus sp. n. While P. bischoffi mainly occurs from Southern Europe to Western Asia, P. helveticus appears to have a more northern distribution in Central Europe. Thus far, the only incidences of syntopy (P. bischoffi, P. helveticus) are from Switzerland.
cording to its body measurements, however, it lies in an area of overlap with $P$. gallicus (Fig. 3a, c [H]). Unfortunately, it is the only specimen that was both intact and suitable for molecular analyses.

Distribution. Fig. 11 only shows the distribution of the specimens examined within this study. Other possible records of $P$. helveticus (still under the name "bischoffi") not shown in Fig. 11 include localities in Austria (Gusenleitner 1995: 165; 1998: 497), Belgium (Baugnée 1996), Czech Republic and Slovakia (Dvořák et al. 2006), France (Graf 1961), Germany (Mauss 2001, Schmid-Egger and Treiber 1989), and Luxembourg (Schneider et al. 1998).

Nevertheless, Fig. 11 indicates a geographical separation between $P$. helveticus sp. n. (in the north) and $P$. bischoffi (in the south), leaving only a small area of overlap. Real syntopy (habitat sharing) between the two species has thus far only been assessed in Switzerland but both species also occur sympatrically in Austria. Furthermore, the verified range of $P$. helveticus sp. n. (Fig. 11) is distinctly smaller than that of $P$. bischoffi, although $P$. helveticus sp. n. is considered to be in a period of expansion (Dvořák et al. 2006, Mauss 2001).

Ecology. In Switzerland, P. helveticus sp. n. is widespread (Fig. 11), usually occurring in wet habitats such as floodplains, fens, bogs, and pits (gravel, sand). The


Figure 12. Drawings of morphological characters used in the key to European paper wasps (Polistes) of the gallicus-group: Mesopleuron with abrupt (a) or gradual (b) sculpture change; female antennae with dark (c) or bright (d) upper side of flagellomeres; male apical flagellum dark (e) or bright (f) on upper side; head in frontal view with black band across clypeus $(\mathbf{g})$ or isolated black spot $(\mathbf{h})$; apical mesosoma in dorsal view with drop-shaped spots on mesoscutum, posterior pronotal stripes ending far from pronotal carina (i) or without drop-shaped spots, pronotal stripes approaching pronotal carina (k); head in dorsal view with genae convex $(\mathbf{I})$ or immediately narrowing $(\mathbf{m})$. Drawings $\mathbf{a}, \mathbf{b}, \mathbf{c}, \mathbf{d}, \mathbf{f}$ are courtesy of H . Cigler, $\mathbf{g}, \mathbf{h}, \mathbf{i}, \mathbf{k}$ of D . Lawniczak, and $\mathbf{e}, \mathbf{1}, \mathrm{m}$ of CSCF.
altitudinal records range from 200 m above sea level (Le Champ-près-Froges, France) for a female (individual RN0378) to 980 m a.s.l. (Muggio, Canton of Ticino, CH) for a male (RN0387). The seasonal records range from 02 April (Saint-Blaise, CH) for a female to 24 November (Gnadental, Germany) for a female (RN0283), but most individuals of both sexes are recorded in July and August (CSCF, in litt.). The earli-
est record for a male is on 21 July (Jestetten, Germany), the latest (RN0157) on 01 October (Courroux, CH). Nests are usually attached below 40 cm to vertical stems ( $\varnothing$ $2-3 \mathrm{~cm}$ ) of reed, alder, willow and other plants (Kofler 2005; Neumeyer et al. 2011).

Etymology. The Latin adjective "helveticus -a -um" means Swiss. The name Polistes helveticus sp. n. pays tribute to the country where the species was first discovered, when a female (Theodor Steck leg., NHMB coll.) was found in Bätterkinden (canton of Berne) in August 1882.

Material examined. Holotype $q$ (RN0139): SWITZERLAND, CANTON ZURICH, Schwerzenbach, Böschen, $47^{\circ} 22^{\prime} 21.67^{\prime \prime} \mathrm{N}, 08^{\circ} 40^{\prime} 01.43^{\prime \prime} \mathrm{E}, 436 \mathrm{~m}, 11 \mathrm{Aug}$ 2010, fen rotation fallow, Rainer Neumeyer leg., NMBE coll.

Original Paralectotype (Weyrauch 1939: 164) of P. bischoffi Weyrauch, 1937: 1 § (RN0325): SWITZERLAND, CANTON ZURICH, Glattbrugg, 8 Sep 1937, Wolfgang Weyrauch leg., HUMCZ coll., labeled as follows: 1. "W. Weyrauch" [printed], "Glattbrugg bei Zürich, 8.9.37" [handwritten]. 2. "Polistula bischoffi Weyrauch 1938" [handwritten]; 3. "Glattbrug [sic] nr Zurich Switzerland [handwritten; most probably added posteriorly]; 4. "Polistes bischoffi § Weyr. paratype." [handwritten; probably added posteriorly].

Paratypes: 1 q (RN0412): AUSTRIA, BURGENLAND, Stadtschlaining, Goberling, 18 May 1990, Michael Madl leg., NHMW coll.; 1 \& (RN0399): CZECH REPUBLIC, SOUTH BOHEMIA, Stachy, Úbislav, 12 Oct 2005, village, Libor Dvořák leg., LD coll.; 1 đ (RN0398): Vlkov nad Lužnicí, 18 Sep 2004, P. Bogusch leg., LD coll.; 1 q (RN0378): FRANCE, ISĖRE, Le Champ-près-Froges, 200 m, 15 Aug 2003, sand pit, J. \& I. Smit leg., JS coll.; 1 Q (RN0282) + $1 \oint^{\Uparrow}$ (RN0284): GERMANY, BADEN-WÜRTTEMBERG, Blaustein, Lautertal westl. Weidach, 30 Aug 1988, Volker Mauss leg., RN coll.; 1 q (RN0362): Kaiserstuhl, 30 Jun 1938, J.P. Wolf leg., ETHZ coll.; 1 q (RN0283): Michelfeld, Gnadental, 24 Nov 1992, Volker Mauss leg., RN coll.; 1 q (RN0388): BAVARIA, Weichs, Weichser Moos, $48^{\circ} 22^{\prime} 55.2^{\prime \prime N}$ N, $11^{\circ} 25^{\prime} 58.8^{\prime \prime} \mathrm{E}$, fen, 31 Jul 1991, Stephan Blank leg., SDEI coll.; 1 § (RN0162): PRINCIPALITY OF LIECHTENSTEIN, Ruggell, $47^{\circ} 13^{\prime} 56.98^{\prime \prime} \mathrm{N}, 09^{\circ} 39^{\prime} 43.61^{\prime \prime} \mathrm{E}$, 444 m, 27 Aug 1996, dam, Simon Bieri leg., ETHZ coll.; 1 ठ (RN0164): Schaan, $47^{\circ} 11^{\prime} 02.53^{\prime \prime N}, 09^{\circ} 31^{\prime} 29.11^{\prime \prime} \mathrm{E}, 455 \mathrm{~m}, 24$ Sep 1997, Simon Bieri leg., ETHZ coll.; 1 \& (RN0400): SLOVAKIA, TRENČÍN REGION, Bzince pod Javorinou, 06 Aug 2004, glade, Libor Dvorák leg., LD coll.; 1 q (RN0230): SWITZERLAND, CANTON BASLE-CITY, Basel, Badischer Bahnhof, $47^{\circ} 34^{\prime} 50.12^{\prime \prime} \mathrm{N}, 07^{\circ} 36^{\prime} 07.63^{\prime \prime} \mathrm{E}, 255$ m, 18 Aug 1995, ruderal field, Rainer Neumeyer leg., RN coll.; 1 \& (RN0377): CANTON BERNE, Bern, 22 Jul 1883, Theodor Steck leg., NMBE coll.; 1 \& (RN0374) + 2 ō (RN0375, RN0376): Bätterkinden, 24 Aug 1887, Theodor Steck leg., NMBE coll.; $1 \sigma^{\top}$ (RN0161): Gampelen, Seewald, 16 Aug 1994, Richard Vernier leg., MHNN
 $07^{\circ} 06^{\prime} 34.74^{\prime \prime} \mathrm{E}, 430 \mathrm{~m}, 22$ Aug 2003, Christian Monnerat leg., MHNN coll.; 1 ठ (RN0157): Courroux, Le Quenet, $47^{\circ} 22^{\prime} 46.78^{\prime \prime N}$, $07^{\circ} 21^{\prime} 40.20^{\prime \prime} \mathrm{E}, 510 \mathrm{~m}, 01$ Oct 2004, Christian Monnerat leg., MHNN coll.; $2 \widehat{O}^{\text {(RN }}$ (RN0155, RN0159): Courtemaîche, La Colombière, $47^{\circ} 27^{\prime} 48.87^{\prime \prime} \mathrm{N}, 07^{\circ} 02^{\prime} 59.38^{\prime \prime} \mathrm{E}, 390 \mathrm{~m}, 22$ Aug 2003, Christian

Monnerat leg., MHNN coll.; 1 § (RN0386): CANTON TICINO, Meride, Fontana, $45^{\circ} 53^{\prime} 44^{\prime \prime} \mathrm{N}, 08^{\circ} 56^{\prime} 46^{\prime \prime} \mathrm{E}, 595 \mathrm{~m}, 24$ Aug 1997, Ladislaus Rezbanyai-Reser leg., NML coll.; $1 \sigma^{\top}$ (RN0387): Muggio, Muggiasca, $45^{\circ} 54^{\prime} 39^{\prime \prime} \mathrm{N}, 09^{\circ} 01^{\prime} 21^{\prime \prime} \mathrm{E}, 980 \mathrm{~m}, 16$ Aug 1996, Ladislaus Rezbanyai-Reser leg., NML coll.; 1 ㅇ (RN0394): CANTON VALAIS, Brig, 09 Aug 1916, anon. leg., ETHZ coll.; 2 § (RN0198, RN0199): CANTON VAUD, Bussigny-près-Lausanne, 16 Aug 1962, Robert Matthey leg., MZL coll.; 1 q (RN0360) + 1 § (RN0361): Lausanne, Aug 1942, Jacques de Beaumont leg., MFNB coll.; 3 ő (RN0200, RN0203, RN0204): Lausanne, Vidy, 02 Aug 1943, Jacques de Beaumont leg., MZL coll.; 1 Q (RN0046): Noville, Longes Rayes, $46^{\circ} 23^{\prime} 21.32^{\prime \prime} \mathrm{N}$, $06^{\circ} 53^{\prime} 31.03^{\prime \prime} \mathrm{E}, 273 \mathrm{~m}, 23$ Aug 2011, shrubberies, Rainer Neumeyer leg., RN coll.; 5 q (RN0047, RN0048, RN0049, RN0050, RN0051): $46^{\circ} 23^{\prime} 14.28^{\prime \prime} \mathrm{N}, 06^{\circ} 53^{\prime} 34.66^{\prime \prime} \mathrm{E}$, 273 m, 23 Aug 2011, tall herbaceous vegetation, Rainer Neumeyer leg., RN coll.; 1 § (RN0160): Saint-Livres, Les Mossières, $46^{\circ} 32^{\prime} 02.47$ "N, $06^{\circ} 21^{\prime} 55.82^{\prime \prime} \mathrm{E}, 700 \mathrm{~m}, 21$ Aug 2002, Christian Monnerat leg., MHNN coll.; 2 § (RN0201, RN0202): SaintSulpice, Jul 1943, Jacques de Beaumont leg., MZL coll.; 1 § (RN0114): Villars-sousYens, Arborex, $46^{\circ} 30^{\prime} 11.03^{\prime \prime N}, 06^{\circ} 25^{\prime} 12.11$ "E, $510 \mathrm{~m}, 25$ Aug 2010, fen, Christian Monnerat leg., MHNN coll.; 2 q (RN0277, RN0278): CANTON ZUG, Hünenberg, Rüssspitz, $47^{\circ} 14^{\prime} 09.40 " \mathrm{~N}, 08^{\circ} 24^{\prime} 39.49^{\prime \prime} \mathrm{E}, 389 \mathrm{~m}, 10 \mathrm{Jul} 2012$, fen, Rainer Neumeyer leg., ETHZ coll.; 1 q (RN0279): $47^{\circ} 14^{\prime} 17.60^{\prime \prime} \mathrm{N}, 08^{\circ} 24^{\prime 27.75 " E, ~} 389 \mathrm{~m}$, 20 Aug 2012, fen, Rainer Neumeyer leg., AMNH coll.; 1 \& (RN0275): CANTON ZURICH, Bauma, Fischbach, $47^{\circ} 23^{\prime} 00.66^{\prime \prime N}$, $08^{\circ} 50^{\prime} 48.30$ "E, $660 \mathrm{~m}, 04 \mathrm{Jul} 2012$, abandoned pit, Rainer Neumeyer leg., RN coll.; 2 q (RN0167, RN0168): 08 Aug 2012, abandoned pit, Rainer Neumeyer leg., CSE coll.; 1 đ (RN0152): Mönchaltorf, Seewisen, $47^{\circ} 19^{\prime} 17.63^{\prime \prime N}, 08^{\circ} 41^{\prime} 55.58^{\prime \prime} \mathrm{E}, 436 \mathrm{~m}, 21$ Aug 2010, fen rotation fallow, Rainer Neumeyer leg., AMNH coll.; 1 \& (RN0003): Pfäffikon, Auslikon, $47^{\circ} 20^{\prime} 42.05^{\prime \prime} \mathrm{N}, 08^{\circ} 47^{\prime} 52.78^{\prime \prime} \mathrm{E}, 539 \mathrm{~m}, 20$ Jun 2011, fen, Rainer Neumeyer leg., RN coll.; 3 q (RN0018, RN0019, RN0020): $47^{\circ} 20^{\prime} 46.9^{\prime \prime} \mathrm{N}, 08^{\circ} 47^{\prime} 50.38^{\prime \prime} \mathrm{E}, 539 \mathrm{~m}, 10$ Aug 2011, fen, Rainer Neumeyer leg., RN coll.; 1 \& (RN0078): Pfäffikon, Irgenhuserriet, $47^{\circ} 20^{\prime} 59.15^{\prime \prime} \mathrm{N}, 08^{\circ} 47^{\prime} 49.98^{\prime \prime} \mathrm{E}, 539 \mathrm{~m}, 06$ Sep 2011, fen, Rainer Neumeyer leg., RN coll.; 2 q (RN0033, RN0034): Seegräben, Schlachtmad, $47^{\circ} 20^{\prime} 23.35^{\prime \prime} N$, $08^{\circ} 46^{\prime} 36.56^{\prime \prime}$ E, $537 \mathrm{~m}, 19$ Aug 2011, fen, Rainer Neumeyer leg., RN coll.; 1 q + 2 ठ (RN0138, RN0153, RN0154): Schwerzenbach, Böschen, $47^{\circ} 22^{\prime} 21.67^{\prime \prime} \mathrm{N}$, $08^{\circ} 40^{\prime} 01.43^{\prime \prime} \mathrm{E}, 436 \mathrm{~m}$, same nest as holotype, 11 Aug 2010, fen rotation fallow, Rainer Neumeyer leg., NMBE coll.; 1 q (RN0276): Weiach, Rüteren, $47^{\circ} 34^{\prime} 03.21^{\prime \prime} \mathrm{N}$, $08^{\circ} 26^{\prime} 44.70^{\prime \prime} \mathrm{E}, 365 \mathrm{~m}, 02$ Apr 2005, gravel pit, Rainer Neumeyer leg., RN coll.; 1 ㅇ (RN0035): Wetzikon, Agerstenriet, $47^{\circ} 20^{\prime} 06.75^{\prime \prime N}, 08^{\circ} 46^{\prime} 57.43^{\prime \prime} \mathrm{E}, 538 \mathrm{~m}, 19$ Aug 2011, fen, Rainer Neumeyer leg., RN coll.; 2 Q (RN0012, RN0013): Wetzikon, Seeriet, $47^{\circ} 20^{\prime} 30.24^{\prime \prime} \mathrm{N}, 08^{\circ} 47^{\prime} 10.89^{\prime \prime} \mathrm{E}, 538 \mathrm{~m}, 05$ Aug 2011, fen, Rainer Neumeyer leg., RN coll.; 1 q (RN0081): 09 Sep 2011, fen, Rainer Neumeyer leg., RN coll.; 1 ㅇ (RN0014): Wetzikon, Robenhuserriet, $47^{\circ} 20^{\prime} 19.16^{\prime \prime} \mathrm{N}, 08^{\circ} 47^{\prime} 02.75^{\prime \prime} \mathrm{E}, 538 \mathrm{~m}$, 05 Aug 2011, fen, Rainer Neumeyer leg., RN coll.; 1 q (RN0017): $47^{\circ} 20^{\prime} 16.20 " \mathrm{~N}$, $08^{\circ} 47^{\prime} 34.35^{\prime \prime} \mathrm{E}, 539 \mathrm{~m}, 05$ Aug 2011, fen, Rainer Neumeyer leg., RN coll.; 1 ő (RN0163): Zürich, < 1900, anon. leg., ETHZ coll.

## c) Key to species of the Polistes gallicus-group

The following dichotomous key only applies to the described species of the gallicusgroup. The text denotes diagnostic traits. However, traits described after a hyphen (-) are those that apply in most cases to species in that half of the couplet, but that may also apply to some species in the alternative half of the same couplet. To determine all European species of Polistes including the dominuld-group, we recommend the keys of Mauss and Treiber (2004), Dvořák and Roberts (2006), and Witt (2009: 129-132), whereby Mauss and Treiber (2004) apply to Germany only.
Q Antenna with 10 flagellomeres. Metasoma with 6 terga ..... 1
§ Antenna with 11 flagellomeres. Metasoma with 7 terga ..... 6
Females:

1 Malar space usually black, mandible spotted yellow (Fig. 12g, h). If yellow area present on malar space, then always smaller than area on mandible2

- Malar space yellow, mandible usually black. If yellow area on mandible, then always smaller than area on malar space dominula-group
2 Flagellum black on upper side, bright yellow to orange on lower side (Fig. 12c). - Metacoxa black. Mesoscutum usually without yellow spot (Fig. 12k). Clypeus with central, black spot (Fig. 12h) or more often with black, horizontal bar (Fig. 12g). Hypopygium entirely black3
- Flagellum bright yellow to orange (Fig. 12d) or faintly darkened on upper side, but never black4

3 Epicnemial carina distinct, marking an abrupt change in sculpture between coarse mesepisternum and smooth epicnemium (Fig. 12a). Malar space : lateral ocelli distance 1.22-1.76. - Mesoscutum with relatively long pubescence (Fig. 12a)
P. biglumis

- Epicnemial carina reduced (Fig. 12b) or absent. Change in sculpture between mesepisternum and epicnemium often gradual (Fig. 12b). Malar space : lateral ocelli distance 0.87-1.19 P. helveticus sp. n .

4 Epicnemial carina reduced (Fig. 12b) or absent. Change in sculpture between mesepisternum and epicnemium frequently gradual (Fig. 12b). Terminal flagellomere length : malar space 1.00-1.24. - Clypeus yellow, although almost never entirely so, frequently with central black spot (Fig. 12h), occasionally even with horizontal black band (Fig. 12g). Mesoscutum usually without yellow spot (Fig. 12k), only occasionally with pair of yellow spots (Fig. 12i). Metacoxa frequently black, only occasionally spotted yellow. Hypopygium entirely black I. bischoffi

- Epicnemial carina distinct (Fig. 12a) or reduced, usually marking a sudden change in sculpture between coarse mesepisternum and smooth epicnemium (Fig. 12a). Terminal flagellomere length : malar space 0.74-1.11. - Clypeus
yellow, with or without central, black spot (Fig. 12h), but almost never with horizontal black band. Metacoxa frequently spotted yellow, only occasionally black5

5 Hypopygium entirely black, only rarely spotted yellow at tip. Lateral part of propodeum with yellow spot usually more than half the size of mesopleural spot (Fig. 6c). Mesoscutum frequently with pair of yellow spots (Fig. 12i). Pronotum with paired longitudinal yellow stripes along posterior margin usually not reaching yellow cross stripe on pronotal collar (Fig. 12i)
P. gallicus

- Hypopygium frequently orange and yellow at tip, only occasionally entirely black. Lateral part of propodeum with yellow spot of less than half the size of mesopleural spot, if present at all. Mesoscutum only occasionally with pair of yellow spots. Pronotum with longitudinal yellow stripes often reaching yellow cross stripe on pronotal collar (Fig. 12k).
P. hellenicus

Males:

6 Gena convex in dorsal view (Fig. 12l) .......................................................... 7

- Gena converging in dorsal view (Fig. 12m)................................................. 8
$7 \quad$ Flagellum black on dorsal side, bright yellow to orange on ventral side (Fig. 12e). Frontal groove and lateral clypeal ridges reduced or absent. Terminal flagellomere length : terminal flagellomere breadth 1.64-2.68. - Black area of upper frons often with bat-shaped yellow spot
.P. biglumis
- Flagellum bright yellow to orange all around (Fig. 12f) in most species. If not, frontal groove and lateral clypeal ridges distinct, and terminal flagellomere length : terminal flagellomere breadth > 2.5 ...........dominula-group (in part)
8 Epicnemium and mesosternum black (Fig. 8), seldom with pair of elongate yellow spots between pro- and mesocoxa ..................................P. hellenicus
- Epicnemium and mesosternum entirely yellow (Fig. 7d) ............................ 9
$9 \quad$ Flagellum black or blackish dorsally, bright yellow to orange ventrally (Fig. 12e)
- Flagellum entirely bright yellow to orange (Fig. 12f) or faintly darkened dorsally. - Frontal groove reduced 11
10 Frontal groove and lateral clypeal ridges reduced. Terminal flagellomere length : terminal flagellomere breadth 1.83-2.78. $\qquad$ P. helveticus sp. n.
- Frontal groove and lateral clypeal ridges very distinct. Terminal flagellomere length : terminal flagellomere breadth $>2.5$.......dominula-group (associus)
11 Clypeus with moderate but distinct lateral ridges. Mesoscutum often with pair of drop-shaped yellow spots (Fig. 12i). Metacoxa frequently spotted yellow dorsally. Terminal flagellomere length : malar space 1.28-2.21 ..... P. gallicus
- Clypeus with reduced or absent lateral ridges. Mesoscutum seldom with pair of drop-shaped yellow spots. Metacoxa seldom spotted yellow dorsally. Terminal flagellomere length : malar space 1.93-2.75

Although it probably belongs to the gallicus-group too, the ambiguous taxon from Greece (and possibly elsewhere) referred to as "Polistes sp. aff. gallicus" is not included in this key because there was not enough material available to examine.

## Discussion

Status of Polistes bischoffi and Polistes helveticus sp. n. Our study unambiguously demonstrates that two distinct species are included within what has been so far considered as Polistes bischoffi Weyrauch, 1937: a light colored species (Polistes bischoffi) with a Southern European to West Asian distribution, and a dark, Central European species described here as Polistes helveticus sp. n.

The distinctivness of these taxa ( $P$. bischoff, $P$. helveticus sp. n .) is revealed in analyses of two independent molecular markers (COX1, ITS1), as well as in our morphometric analyses. Moreover, $P$. helveticus sp. n. is probably closely related to $P$. bischoffi (as suggested by the reduced epicnemial carina and the association with wetlands) and occurs in the same habitats, sometimes syntopically, but appears not to interbreed (Neumeyer et al. 2011). Taken together, these results suggest that three independent criteria are met to reveal the presence of a new species: molecules, morphology, and syntopy without interbreeding (Neumeyer et al. 2011).

The unnoticed presence of a cryptic species in Europe is surprising and calls for an explanation. Interestingly, the first record for Polistes bischoffi Weyrauch, 1937 (rev. status) in Switzerland refers to two individuals (RN0170, RN0171) found in 1927 in Versoix near Geneva, in the extreme southwest of Switzerland where Polistes gallicus is known to have occurred before 1900 (cf. our examined individual RN0208). The second Swiss record (RN0156) of P. bischoffi is from Chabrey on Lake Neuchâtel in 1992, and the third (RN0169) from Regensdorf near Zurich in 1997, all together suggesting a recent range expansion from the southwestern to the northeastern part of the Swiss midlands, where P. gallicus still does not occur. We hypothesize that P. bischoffi was originally present but remained undetected within the range of the superficially similar $P$. gallicus, and became conspicuous only after it expanded beyond the range of P. gallicus, possibly due to global warming.

Morphometry. By applying multivariate ratio analysis (MRA) most taxa of the gallicus-group are rather well differentiated (Fig. 3), with the exception of P. gallicus and $P$. hellenicus. The use of further measurements may have resulted in better differentiation between these two species, as the addition of characters has indeed improved the separation of sibling species in some other Hymenoptera (e.g., Kenis and Mills 1998, Villemant et al. 2007). However, such analyses are beyond the scope of this study, as P. bischoffi and P. helveticus sp. n. were clearly separated by the first shape PC (Fig. 4).

The latter two species were of comparable size, so allometric variation did not interfere with the interpretation of the data. This was also unlikely to bias the differentiation of $P$. biglumis, although this large species accounted for the rather strong
correlative pattern between size and shape in the shape PCA of all five species of the gallicus-group (Figs 3c, d). Since P. biglumis could clearly be separated from the rest of the gallicus-group by qualitative morphological characters and molecular analysis, the morphometric separation was assumed to be based on "true" shape differences and not merely on an indirect size effect. Hence, we see no need to correct the best separating ratio (Tab. 5) for allometric size effects, although such a procedure has sometimes been suggested (Janzon 1986, Seifert 2008, Bartels et al. 2011).

The LDA ratio extractor revealed ratios that separated some of the species with very little or no overlap (Table 5, species comparisons marked with *). It is noteworthy that these ratios were composed of measurements from widely separated body parts; for instance metatibia length (tib3.l) to malar space (msp.l) was the best ratio for separating the females of $P$. helveticus sp. n. from $P$. bischoff. This is in contrast to more commonly used ratios that are calculated from measurements of the same or adjacent body parts, such as eye length to breadth or clypeus height to breadth (e.g., Arens 2011). In our study, such standard ratios are clearly less powerful for separating taxa (compare PCA ratio spectra in Fig. 5), an observation that was also made by László et al. (2013) in their application of MRA to parasitic wasps.

Utility of the molecular markers. An important question when using molecular markers to separate closely related species, is whether a clear gap (the barcoding gap) exists between "within-species" distances and "between-species" distances. Buck et al. (2012)'s detailed study of the Nearctic Fuscopolistes revealed no barcoding gap within this group for COX1. In fact, half of the species included in their study showed a "negative barcoding gap", i. e. a situation where "the maximum intraspecific divergence was greater than the distance to the nearest neighbour from another species" (Buck et al. 2012: 34). In our case, the evaluation of such a barcoding gap would strongly depend on our interpretation of the two clades found within $P$. dominula with the mitochondrial marker. Two hypotheses can be formulated: firstly, two cryptic species may be present in Central Europe; alternatively, two distinct mitochondrial haplotypes may exist within one single species. As P. dominula was not the focus of our study, we did not perform any morphometric analyses for this taxon. The nuclear marker ITS1 did not recover these two clades. As a nuclear DNA marker, ITS1 has a lower rate of mutation than the mitochondrial marker, as indicated by the overall smaller genetic distances between species for ITS1 than for COX1. It is therefore possible that ITS1 evolves too slowly to recover the recent divergence between the two clades observed within $P$. dominula. However, ITS1 appeared highly suitable for recovering differences between other closely related species. Therefore, we favor the hypothesis that two mitochondrial haplotypes may coexist in Central Europe within P. dominula, as demonstrated for other species (Avtzis et al. 2008, Arthofer et al. 2010). Possibly, the two haplotypes revealed in $P$. dominula reflect two distinct Pleistocene refugia that have facilitated sequence divergence in the mitochondrial marker; divergence time was presumably not long enough for preventing the populations from successfully interbreeding when they entered in contact again. Our example stresses the importance of using additional criteria (morphometry, nuclear DNA) in addition to one single mitochondrial marker
(e.g., the universal barcode) to examine the status of populations in systematics. Deep within-species divergences in mitochondrial DNA sequences may be more widespread than hitherto assumed, especially when sampling is done over the entire range of a species (Bergsten et al. 2012, Buck et al. 2012).

In conclusion, our study demonstrates the power of the combined use of morphometrics and molecular markers in unraveling cryptic diversity, as proposed under the framework of integrative taxonomy (Schlick-Steiner et al. 2010). It also stresses the importance of using multiple molecular markers to evaluate the status of unclear taxa.

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