RESEARCH ARTICLE



Review of the fish parasitic genus *Ceratothoa* Dana, 1852 (Crustacea, Isopoda, Cymothoidae) from South Africa, including the description of two new species

Kerry A. Hadfield^{1,3,†}, Niel L. Bruce^{2,3,‡}, Nico J. Smit^{3,§}

Department of Zoology, University of Johannesburg, P.O. Box 524, Auckland Park, 2006 South Africa
 Museum of Tropical Queensland, Queensland Museum and School of Marine and Tropical Biology, James Cook University; 70–102 Flinders Street, Townsville, Australia 4810 3 Water Research Group (Ecology), Unit for Environmental Sciences and Management, Potchefstroom Campus, North West University, Private Bag X6001, Potchefstroom, 2520, South Africa

http://zoobank.org/F55EBCB7-CFFE-41C4-AFE8-78BD75341291
 http://zoobank.org/9DA13F3D-AB3A-43E0-BF49-A71EBB8612D7
 http://zoobank.org/9EB3BA08-5E8E-420C-9B3D-F40E308086D8

Corresponding author: Kerry A. Hadfield (kerryh26@yahoo.com)

Academic editor: S. Taiti | Received 19 December 2013 | Accepted 20 March 2014 | Published 10 April 2014 http://zoobank.org/E803925E-0418-463D-863E-183EDDAAA487

Citation: Hadfield KA, Bruce NL, Smit NJ (2014) Review of the fish parasitic genus *Ceratothoa* Dana, 1852 (Crustacea, Isopoda, Cymothoidae) from South Africa, including the description of two new species. ZooKeys 400: 1–42. doi: 10.3897/zooKeys.400.6878

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* **sp. 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: 217–218; 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 percopod 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 *Lobothorax* 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 perconite 1 with an anterolateral ridge and small cephalon sunken into perconite 1. Perconite 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 mm TL; 15 mm W), collected from a trawl (34°26'S, 24°13'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°26'S, 24°13'E) along the south coast of South Africa (30-04-2003), coll. N.J. Smit: Three females (22–26 mm TL; 12–15 mm W), three males (11–13 mm TL; 5–6 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°51'S, 25°52'E), 1978: female (28 mm TL; 12 mm W). Eastern Cape, no date: female (20 mm TL; 11 mm W), male (7 mm TL; 3 mm W). Tsitsikamma Grootbank, Sout River (34°1'S, 23°28'E), September 1993: female (19 mm TL; 8 mm 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 2 0.9 times as long as article 1; article 3 0.3 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 3 1.5 times as long as article 2, as long as wide; article 4 0.8 times as long as wide, 0.8 times as long as article 3; article 5 0.5 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, 1.4 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 1. *Ceratothoa africanae* sp. n. female holotype (29 mm) (SAM A45937): **A** dorsal view **B** anterodorsal view of pereonite 1 and cephalon **C** ventral view of cephalon **D** dorsal view of pleotelson **E** lateral view.



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



Figure 3. *Ceratothoa africanae* sp. n. female holotype (29 mm) (SAM A45937): **A** pereopod 1 **B** pereopod 2 **C** pereopod 6 **D** pereopod 7.



Figure 4. *Ceratothoa africanae* sp. n. female paratype (30 mm) (SAM A45938): A dorsal pleopod 1
B dorsal pleopod 2 C dorsal pleopod 3 D dorsal pleopod 4 E dorsal pleopod 5 F ventral pleopod 1
G ventral pleopod 2 H ventral pleopod 3 I ventral pleopod 4 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 **C** ventral view of cephalon **D** dorsal view of pleotelson **E** lateral view.



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



Figure 7. *Ceratothoa africanae* sp. n. male paratype (14 mm) (SAM A45938): **A** dorsal pleopod 1 **B** dorsal pleopod 2 **C** dorsal pleopod 3 **D** dorsal pleopod 4 **E** dorsal pleopod 5 **F** ventral pleopod 1 **G** ventral pleopod 2 **H** ventral pleopod 3 **I** ventral pleopod 4 **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*; 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°1'S, 23°52'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°1'S, 23°52'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 mm TL; 3, 6 mm W), March 2005, coll. K.A. Hadfield (SAM A45941). From the buccal-cavity of *Sparadon durbanensis*: female (15 mm TL; 6 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°49'S, 20°0'E): female (18 mm TL; 7 mm W), male (9 mm TL; 3 mm W). Kenton-on-sea (33°42'S, 26°41'E): female (14 mm TL; 5 mm W), male (5 mm TL; 2 mm W), May 1974. Morgan Bay (32°42'S; 28°20'E): two females (10, 12 mm TL; 3, 4 mm W), April 2003. Swartkops River Estuary (33°52'S; 25°38'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°22'S, 29°53'E): female (15 mm TL; 6 mm W), male (6 mm TL; 2 mm W), September 1975. Tshani (31°56'S, 29°12'E): female (10 mm TL; 3 mm W), June 1996.

From *Diplodus cervinus hottentotus*: Kleinemonde (33°32'S, 27°03'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°16'S, 27°29'E): female (18 mm TL; 8 mm W), male (9 mm TL; 4 mm W), February 1976; Knysna (34°5'S, 23°3'E): female (19 mm TL; 7 mm W), male (8 mm TL; 3 mm W), 1945–1969. Tsitsikamma National Park (34°1'S, 23°52'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°46'S, 26°28'E): four pullus (5 mm TL; 2 mm W), July 1975. Kleinemonde (33°32'S, 27°03'E): five pullus (4 mm TL; 2 mm W), February 1977. Knysna (34°5'S, 23°3'E): two pullus (5 mm TL; 2 mm W), 1945–1965. Tsitsikamma National Park (34°1'S, 23°52'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. Eves 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 2 0.8 times as long as article 1; article 3 0.3 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 3 1.4 times as long as article 2, 1.1 times as long as wide; article 4 1.1 times as long as wide, 0.9 times as long as article 3; article 5 0.5 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 0.8 times 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 **C** ventral view of cephalon **D** dorsal view of pleotelson **E** lateral view.



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



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



Figure 11. Ceratothoa famosa sp. n. female paratype (26 mm) (SAM A45941): A dorsal pleopod 1
B dorsal pleopod 2 C dorsal pleopod 3 D dorsal pleopod 4 E dorsal pleopod 5 F ventral pleopod 1
G ventral pleopod 2 H ventral pleopod 3 I ventral pleopod 4 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 **C** ventral view of cephalon **D** dorsal view of pleotelson **E** lateral view.



Figure 13. *Ceratothoa famosa* sp. n. male paratype (12 mm) (SAM A45941): **A** antennule **B** antenna **C** maxillule **D** mandible **E** maxilla **F** maxilliped **G** uropod **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 **C** dorsal pleopod 3 **D** dorsal pleopod 4 **E** dorsal pleopod 5 **F** ventral pleopod 1 **G** ventral pleopod 2 **H** ventral pleopod 3 **I** ventral pleopod 4 **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 P1–P4 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 mm TL; 16 mm 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 15. *Ceratothoa imbricata* (Fabricius, 1775), female holotype (34 mm) (BMNH 1979.403.1): **A** dorsal view **B** antero-dorsal view of pereonite 1 and cephalon **C** dorsal view of pleotelson **D** lateral view.



Figure 16. *Ceratothoa imbricata* (Fabricius, 1775), female holotype (34 mm) (BMNH 1979.403.1): **A** pereopod 1 **B** pereopod 2 **C** pereopod 6 **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 **C** ventral view of cephalon **D** dorsal view of pleotelson **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 **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 a, 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 a, 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. huttoni* 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 **B** antero-dorsal view of pereonite 1 and cephalon **C** dorsal view of pleotelson **D** lateral view.



Figure 20. *Ceratothoa trigonocephala* (Leach, 1818), female lectotype (42 mm) (NHMUK 2013.1013): **A** pereopod 1 **B** pereopod 2 **C** pereopod 6 **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.

Acknowledgements

The financial assistance of the National Research Foundation (NRF) towards this research is hereby acknowledged (project IFR2011040100022). Opinions expressed and conclusions arrived at, are those of the authors and are not necessarily to be attributed to the NRF. We thank the Captain and crew of the FRS *Africana* and Dr Sharon du Plessis (MCM Chief Scientist) for the opportunity to collect material during the April/ May 2003 South Coast Hake Biomass Survey. Special thanks to the South African Institute for Aquatic Biodiversity (SAIAB) for allowing us to sample their fish collection and obtain valuable data on these isopods. Further thanks to Liz Hoenson from the Iziko South African Museum, Cape Town (SAM) and Miranda Lowe from the Natural History Museum, UK (NHMUK) for loan of the museum material used. NLB thanks the Museum of Tropical Queensland for permission to travel for this body of work.

References

- Adlard RD, Lester RJG (1994) Dynamics of the interaction between the parasitic isopod, Anilocra pomacentri, and the coral reef fish, Chromis nitida. Parasitology 109: 311–324. doi: 10.1017/S0031182000078343
- Anonymous (1999) International Code of Zoological Nomenclature (Fourth Edition). International Commission on Zoological Nomenclature, London, 306 pp.
- Barnard KH (1925) Contributions to the crustacean fauna of South Africa. No 9. Further additions to the list of Isopoda. Annals of the South African Museum 20: 381–412.
- Barnard KH (1940) Contributions to the crustacean fauna of South Africa. 12. Further additions to the Tanaidacea, Isopoda, and Amphipoda, together with keys for the identification of the hitherto recorded marine and freshwater species. Annals of the South African Museum 32: 381–543.

- Bianconi G (1869) Specimina zoologica Mosambicana, Fasciculus XVII. Memorie dell'Accademia delle Scienze dell'Istituto di Bologna 9: 199–222.
- Bleeker P (1857) Recherches sur les Crustacés de L'Inde Archipelagique. II. Sur les Isopodes Cymothoadiens de L'Archipel Indien. Natuurkundige vereeniging in Nederlandsche-Indie, Batavia, Verhandelingen, 2: 20–40.
- Bowman TE (1978) Nomenclatural problems in the cymothoid isopod genera *Ceratothoa*, *Codonophilus*, *Glossobius* and *Meinertia* – their solution by applying the law of priority. Crustaceana 34: 217–219. doi: 10.1163/156854078X00754
- Bragoni R, Romestand B, Trilles J-P (1984) Parasitoses à cymothoadien chez le loup (*Dicentrarchus labrax* Linnaeus, 1758) en élevage. I. Écophysiologie parasitaire dans le Cas de l'Étang de Diana (Haute-Corse) (Isopoda, Cymothoidae). Crustaceana 47: 44–51. doi: 10.1163/156854084X00306
- Brandt A, Poore GCB (2003) Higher classification of the flabelliferan and related Isopoda based on a reappraisal of relationships. Invertebrate Systematics 17: 893–923. doi: 10.1071/ IS02032
- Brown RW (1956) Composition of Scientific Words. Smithsonian Institution Press, Washington, D.C., 863 pp.
- Bruce NL, Bowman TE (1989) Species of the parasitic isopod genera *Ceratothoa* and *Glossobius* (Crustacea: Cymothoidae) from the mouths of flying fishes and halfbeaks (Beloniformes). Smithsonian Contributions to Zoology 489: 1–28.
- Bruce NL, Ton HML, Poore GCB (2002) Cymothoidae Leach, 1814. In: Poore GCB (Ed) Crustacea: Malacostraca: Syncarida, Peracarida: Isopoda, Tanaidacea, Mictacea, Thermosbaenacea, Spelaeofriphacea. Zoological Catalogue of Australia, Volume 19.2A, CSIRO Publishing, Melbourne, 434 pp.
- Brusca RC (1981) A monograph on the Isopoda Cymothoidae (Crustacea) of the eastern Pacific. Zoological Journal of the Linnean Society 73: 117–199. doi: 10.1111/j.1096-3642.1981.tb01592.x
- Coleman CO, Lowry JK, Macfarlane T (2010) DELTA for beginners. An introduction into the taxonomy software package DELTA. ZooKeys 45: 1–75. doi: 10.3897/zookeys.45.263
- Dana JD (1852) On the classification of the Crustacea Choristopoda or Tetradecapoda. American Journal of Sciences and Arts 2: 297–316.
- Dana JD (1853) Crustacea. Part II. United States Exploring Expedition during the years 1838, 1839, 1840, 1841, 1842, under the command of Charles Wilkes, USN. C. Sherman, Philadelphia, 696–805.
- Ellis JP (1981) Some type specimens of Isopoda (Flabellifera) in the British Museum (Natural History), and the isopods in the Linnaean Collection. Bulletin of the British Museum (Natural History) 40: 121–128.
- Fabricius JC (1775) Systema Entomologiae, sistens Insectorum Classes, Ordines, Genera, Species, adiectis Synonymis, Locis, Descriptionibus, Observationibus. Officina Libraria Kortii, Flensburgi & Lipsiae, 832 pp. doi: 10.5962/bhl.title.36510
- Fabricius JC (1787) Mantissa Insectorum sistens eorum species nuper detectas adiectis characteribus genericus, differentiis specificis, emendationibus, observationibus. Christ. Gottl. Proft., Hafniae, 348 pp. doi: 10.5962/bhl.title.36471

- Fabricius JC (1793) Entomologia Systematica emendata et acuta, secundum classes, ordines, genera, species adjectis synonimis, locis, observationibus, descriptionibus. Christ. Gottl. Proft., Hafniae, 519 pp.
- Filhol H (1885) Mission de Isle Campbell. Recherches zoologiques, botaniques, et géologiques faites a l'Isle Campbell et en Nouvelle-Zélande. Recueil de Mémoires, Rapports et Documents relatifs a l'observation du passage de Vénus sur le soleil du 9 Décembre, 1874. Libraire des Compte rendus de séances de l'Académie des Sciences, Paris, 1–182, pl. 135.
- Froese R, Pauly D (Eds) (2013) FishBase. World Wide Web electronic publication, version (09/2009). http://www.fishbase.org [accessed May 2013]
- Guérin-Méneville FÉ, Cuvier G (1829–1843) Iconographie du règne animal de G. Cuvier; ou, Représentation dáprès nature de lúne des espèces les plus remarquables, et souvent non encore figurées, de chaque genre dánimaux. Avec un texte descriptif mis au courant de la science. Ouvrage pouvant servir d'atlas à tous les traités de zoologie. Crustaces. J. B. Baillière, Paris, 48 pp.
- Hadfield KA (2012) The biodiversity and systematics of marine fish parasitic isopods of the family Cymothoidae from southern Africa. PhD thesis, University of Johannesburg, Johannesburg, South Africa, 445 pp.
- Hadfield KA, Bruce NL, Smit NJ (2010) Redescription of the monotypic genus *Cinusa* Schioedte and Meinert, 1884 (Isopoda, Cymothoidae), a buccal-cavity isopod from South Africa. Zootaxa 2437: 51–68.
- Hadfield KA, Bruce NL, Smit NJ (2011) *Cymothoa hermani* sp. n. (Isopoda, Cymothoidae, Crustacea), a parasitic isopod, collected off the Zanzibar coast, Tanzania from the mouth of a parrotfish (Scaridae). Zootaxa 2876: 57–68.
- Hadfield KA, Bruce NL, Smit NJ (2013) Review of the fish-parasitic genus *Cymothoa* Fabricius, 1783 (Isopoda, Cymothoidae, Crustacea) from the south-western Indian Ocean, including a new species from South Africa. Zootaxa 3640: 152–176. doi: 10.11646/ zootaxa.3640.2.2
- Hadfield KA, Bruce NL, Szinetár C, Smit NJ (in press) *Ceratothoa retusa* (Schiœdte & Meinert, 1883) (Isopoda, Cymothoidae), a variable species of fish parasitic marine isopod from the Indian Ocean. Crustaceana.
- Hale HM (1926) Review of Australian isopods of the cymothoid group. Part II. Transactions of the Royal Society of South Australia 50: 201–234, pls. 226, 227.
- Hale HM (1927) The fauna of Kangaroo Island, South Australia. 1. The Crustacea. Transactions and proceedings of the Royal Society of South Australia 51: 307–321.
- Hale HM (1929) The Crustaceans of South Australia. Part II. Weir, Government Printer, Adelaide, 202–380.
- Hale HM (1937) Isopoda and Tanaidacea. Australasian Antarctic Expedition 1911–14 Under the leadership of Sir Douglas Mawson, OBE, BE, DSc, FRS Scientific Reports, Series C– Zoology and Botany 2: 1–45.
- Hale HM (1940) Report on the cymothoid Isopoda obtained by the F.I.S. "Endeavour" on the coasts of Queensland, New South Wales, Victoria, Tasmania, and South Australia. Transactions of the Royal Society of South Australia 64: 288–304.
- Haswell WA (1881) On some new Australian marine Isopoda. Part I. Proceedings of the Linnean Society of New South Wales 5(4): 470–481.

- Haswell WA (1882) Catalogue of the Australian stalk- and sessile-eyed Crustacea. The Australian Museum, Sydney, 1–324. doi: 10.5962/bhl.title.1948
- Hilgendorf F (1879) Die von Herrn. W. Peters in Moçambique gesammelten Crustaceen. Monatsbericht de Königlich Preussischen Akademie der Wissenschaften zu Berlin, (Physikalisch-mathematischen Klasse) 1878: 782–851.
- Horton T (2000) *Ceratothoa steindachneri* (Isopoda: Cymothoidae) new to British waters with a key to north-east Atlantic and Mediterranean *Ceratothoa*. Journal of the Marine Biological Association of the United Kingdom 80: 1041–1052. doi: 10.1017/S0025315400003106
- Horton T, Okamura B (2001) Cymothoid isopod parasites in aquaculture: a review and case study of a Turkish sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus auratus*) farm. Diseases of Aquatic Organisms 46: 181–187. doi: 10.3354/dao046181
- Jones CM, Miller TL, Grutter AS, Cribb TH (2008) Natatory-stage cymothoid isopods: description, molecular identification and evolution of attachment. International Journal for Parasitology 38: 477–491. doi: 10.1016/j.ijpara.2007.07.013
- Kensley B (1978) Guide to the Marine Isopods of Southern Africa. South African Museum & The Rustica Press, Wynberg, Cape Town, 173 pp.
- Kensley B (2001) Biogeography of the marine Isopoda of the Indian Ocean, with a check-list of species and records. In: Kensley B, Brusca RC (Eds) Isopod Systematics and Evolution Crustacean Issues 13. A.A. Balkema, Rotterdam, 205–264.
- Kensley B, Schotte M (1989) Guide to the Marine Isopod Crustaceans of the Caribbean. Smithsonian Institution Press, Washington, D.C. & London, 308 pp.
- Ketmaier V, Joyce DA, Horton T, Mariani S (2008) A molecular phylogenetic framework for the evolution of parasitic strategies in cymothoid isopods (Crustacea). Journal of Zoological Systematics and Evolutionary Research 46: 19–23.
- Kussakin OG (1979) Marine and brackishwater likefooted Crustacea (Isopoda) from the cold and temperate waters of the Northern Hemisphere. Suborder Flabellifera. Izdatel'stvo Nauka, Leningrad [In Russian], 472 pp.
- Leach WE (1814) Crustaceology. In: Brewster D (Ed) The Edinburgh Encyclopaedia. Baldwin, London, 383–437, pl. 221.
- Leach WE (1818) Cymothoadées. In: Cuvier F (Ed) Dictionnaire des Sciences Naturelles. Strasbourg et Levrault, Paris, 338–354.
- Martin MB, Bruce NL, Nowak BF (2013) Redescription of *Ceratothoa carinata* (Bianconi, 1869) and *Ceratothoa oxyrrhynchaena* Koelbel, 1878 (Crustacea: Isopoda: Cymothoidae), buccal-attaching fish parasites new to Australia. Zootaxa 3683: 395-410.
- Menzies RJ (1962) The zoogeography, ecology, and systematics of the Chilean marine isopods. Reports of the Lund University Chile Expedition 1948–49. 42. Lunds Universitets Årsskrifte, N.F. Avd. 2, 57(11): 1–162.
- Miers EJ (1876) Catalogue of the stalk and sessile-eyed Crustacea of New Zealand. Colonial Museum and Geological Department of New Zealand, National History Publication 10: 1–133.
- Miers EJ (1884) Crustacea. Report on the zoological collections made in the Indo-Pacific Ocean during the voyage of HMS "Alert", 1881–1882. BMNH, London, 299–311.
- Milne Edwards H (1840) Histoire Naturelle des Crustacés Comprenent l'anatomie, la physiologie et la classification de ces animaux. Roret, Paris, 3, i–ii, 638 pp.

- Mladineo I (2002) Prevalance of *Ceratothoa oestroides* (Risso, 1826), a cymothoid isopode parasite, in cultured sea bass *Dicentrarchus labrax* L. on two farms in middle Adriatic Sea. Acta Adriatica 43: 97–102.
- Nierstrasz HF (1915) Die Isopoden-Sammlung im Naturhistorischen Reichsmuseum zu Leiden – 1. Cymothoidae. Zoologische Mededelingen (Leiden) 1: 71–108, pls. 103, 104.
- Nierstrasz HF (1931) Isopoda genuina. II. Flabellifera. In: Weber M, De Beaufort LF (Eds) Die Isopoden der Siboga-Expedition. E.J. Brill, Leiden, 123–233, pls. 110, 111.
- Otto AW (1828) Beschreibung einiger neuen, in den Jahren 1818 und 1819, im Mittellländischen Meere gefundener Crustaceen. Nova Acta Academiae Caesareae Leopoldino Carolinae 14: 331–354.
- Ravi V, Rajkumar M (2007) Effect of isopod parasite, *Cymothoa indica* on gobiid fish, *Oxyurichthys microlepis* from Parangipettai coastal waters (South-east coast of India). Journal of Environmental Biology (India) 28: 251–256.
- Richardson H (1904) Contributions to the natural history of the Isopoda. Proceedings of the United States National Museum 27: 1–89. doi: 10.5479/si.00963801.27-1369.657
- Richardson H (1905) A monograph on the isopods of North America. Bulletin of the United States National Museum 54: vii–liii, 1–727.
- Richardson H (1910) Marine isopods collected in the Philippines by the U.S. Fisheries steamer Albatross in 1907–08. Bureau of Fisheries Document 736: 1–44.
- Pillai NK (1954) A preliminary note on the Tanaidacea and Isopoda of Travancore. Bulletin of the Central Research Institute, University of Kerala, India 3: 1–21.
- Romestand B (1979) Etude ecophysiologique des parasitoses a cymothoadiens. Annales de Parasitologie Humaine et Comparee 54: 423–448, pls.1–4.
- Schioedte JC, Meinert F (1883) Symbolæ ad monographium Cymothoarum crustaceorum familiæ. III. Saophridæ. IV. Ceratothoinæ. Naturhistorisk Tidsskrift, Kjøbenhavn 13: 281–378, pls. 211–286.
- Schioedte JC, Meinert F (1884) Symbolæ ad monographium cymothoarum crustaceorum isopodum familiæ. IV. Cymothoidæ Trib. II. Cymothoinæ. Trib. III: Lironecinæ. Naturhistorisk Tidsskrift, Kjøbenhavn 14: 221–454, pls. 226–213.
- Schultz GA (1969) How to know the marine isopod crustaceans. Wm. C. Brown, Iowa, 359 pp.
- Stebbing TRR (1893) A History of Crustacea. Recent Malacostraca. Kegan Paul, Trench, Trubner & Co. Ltd., London, xvii, 466 pp.
- Stebbing TRR (1900) On Crustacea brought by Dr Willey from the South Seas. In: Willey A (Ed) Zoological results based on material from New Britiain, New Guinea, Loyalty Islands and elsewhere, collected during the years 1895, 1896 and 1897. Cambridge University Press, Cambridge, 605–690.
- Stebbing TRR (1902) South African Crustacea. Part 2. Marine Investigations in South Africa 2: 1–92.
- Stebbing TRR (1908) General catalogue of South African Crustacea. (Part V. of S.A. Crustacea, for the Marine Investigations in South Africa). Annals of the South African Museum 6: 281–594.
- Stebbing TRR (1910a) Isopoda from the Indian Ocean and British East Africa. The Percy Sladen Trust Expedition to the Indian Ocean under the leadership of Mr J. Stanley Gar-

diner. Volume III. Transactions of the Linnean Society of London (Zoology) 14: 83–122, 125–111 pls.

- Stebbing TRR (1910b) General catalogue of South African Crustacea. Part V. of S.A. Crustacea, for the Marine Investigations in South Africa. Annals of the South African Museum 6: 281–594, pls 216–222.
- Stebbing TRR (1911) Indian isopods. Records of the Indian Museum 6: 179–191.
- Trilles J-P (1972) Sur quatre isopodes cymothoides du Pacifique (Nouvelle Caledonie). Cahiers de l'Office de Recherche Scientifiques et Techniques Outre Mers, série Océanographique 10: 3–17.
- Trilles J-P (1973) Les Cymothoidae (Isopoda, Flabellifera) du Muséum national d'Histoire naturelle de Paris. Étude critique accompagnée de précisions en particulier sur la répartition géographique et l'écologie des différentes espèces représentées. I. Les Ceratothoinae Schiœdte & Meinert, 1883. Bulletin du Muséum national d'Histoire naturelle, Paris, 3e série, Zoologie 91: 1231–1268 [for 1972].
- Trilles J-P (1986) Les Cymothoidae (Crustacea, Isopoda, Flabellifera) d'Afrique. Bulletin du Muséum National d'Histoire Naturelle 8: 617–636.
- Trilles J-P (1991) Present researches and perspective on Isopoda (Cymothoidae and Gnathiidae) parasites of fishes (systematics, faunistics, ecology, biology and phyisology). Wiadomosci Parazytologiczne 37: 141–143.
- Trilles J-P (1994) Les Cymothoidae (Crustacea, Isopoda) du Monde. Podrome pour une faune. Studia Marina 21/22: 1–288 [for 1991]
- Trilles J-P (2008) Some marine isopods from the Senckenberg Research Institute (Frankfurt am Main, Germany) (Crustacea, Isopoda: Cymothoidae, Aegidae, Corallanidae, Cirolanidae). Senckenbergiana Biologica 88: 21–28.
- Trilles JP, Ravichandran S, Rameshkumar G (2011) A checklist of the Cymothoidae (Crustacea, Isopoda) recorded from Indian fishes. Acta Parasitologica 56: 446–459. doi: 10.2478/s11686-011-0077-z
- Yu H, Li X (2003) Study on the Cymothoidae from Chinese waters. Studia Marina Sinica 45: 223–238. [Chinese]

RESEARCH ARTICLE



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

Sébastien Lavoué^{1,†}, John P. Sullivan^{2,‡}

Institute of Oceanography, National Taiwan University, Roosevelt Road, Taipei 10617, Taiwan 2 Cornell University Museum of Vertebrates, 159 Sapsucker Woods Road, Ithaca, New York 14850 USA

http://zoobank.org/6DC89A1D-15B4-481F-9369-525CF0162FEB
http://zoobank.org/0D434320-BF66-4BAC-BEA6-BD09841E0581

Corresponding author: Sébastien Lavoué (microceb@hotmail.com)

Academic editor: C. Baldwin H	Received 2 December 2013 Accepted 26 March	2014 Published 10 April 2014
http://		0B132

Citation: Lavoué S, Sullivan JP (2014) *Petrocephalus boboto* and *Petrocephalus arnegardi*, two new species of African electric fish (Osteoglossomorpha, Mormyridae) from the Congo River basin. ZooKeys 400: 43–65. doi: 10.3897/ zookeys.400.6743

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.

Copyright S. Lavoué, J.P. Sullivan. This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

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' 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/silver-chloride 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 1. 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. (gray-filled star) at Odzala-Kokua National Park, Republic of the Congo and *Petrocephalus binotatus* (white-filled 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 bsequences) 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 (GTR+ Γ), 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°N, 24.24°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 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/ 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 mm) with the posterior one very close to eye (distance between posterior nostril and eye = 0.1 mm). Dorsal and anal fins originating in posterior half of body (SL/PDD = 1.6 and SL/PAD = 1.8). Predorsal distance slightly greater than pre-anal distance (PDD/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 mm. Distance between pectoral fin and anal fin = 16.4 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 msec, duration of peak 2 = 0.041 msec, duration of peak 3 = 0.079 msec, total EOD duration = 0.257 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* sp. 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

	-	•				
	<i>P. boboto</i> sp. n.	P. binotatus			P. arne,	gardi sp. n.
	Holotype (u) from	Holotype (m) from	Holotype (m) from	Paratypes f	rom Odzala	Two non-types (u, m) from
	Yangambi	Ikengo	Odzala	Min-Max	Mean (stdev)	Yangambi
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

	<i>P. boboto</i> sp. n.	P. binotatus			P. arneg	<i>cardi</i> sp. n.
	Holotype (u) from	Holotype (m) from	Holotype (m) from	Paratypes from	n Odzala	Two non-types (u, m) from
	Yangambi	Ikengo	Odzala	Min-Max Me:	an (stdev)	Yangambi
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 (HL/IOW = 2.5-3.0, mean = 2.8 and HL/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 (HL/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* sp. 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 mm SL, male, Republic of the Congo: Cuvette Ouest, Congo River basin, Pandaka River, Odzala-Kokua National Park, 0.62°N, 14.92°E, Friel et al., August 2002.

Paratypes (17). Republic of the Congo: Cuvette-Ouest: Congo River basin: CUMV 88076, tag no. 5076, 69.6 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 88031, tag no. 5097, 71.3 mm SL, sex undetermined, same data as holotype; CUMV 88031, tag no. 5097, 71.3 mm SL, sex undetermined, same data as holotype; CUMV 88031, tag no. 5097, 74.8 mm SL, sex undetermined, Lékénie River at Mboko landing, Odzala-Kokua National Park, 0.62°N, 14.90°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°N, 14.90°E, Friel et al., August 2002; CUMV 88065, tag no. 5002, 70.7 mm SL, sex undetermined [cytochrome b gene determined], Lékénie River at Mboko landing, Odzala-Kokua National Park, 0.62°N, 14.90°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°N, 14.90°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°N, 14.90°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°N, 14.90°E, Friel et al., August 2002; CUMV 88123, tag no. 5377, 68.9 mm SL, male, Lékoli River, Odzala-Kokua National Park, 0.61°N, 14.93°E, Friel et al., August 2002; CUMV 87838, tag no. 5404, 90.1 mm SL, male, Lokoué River, Odzala-Kokua National Park, 0.90°N, 15.12°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°N, 15.12°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°N, 14.91°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°N, 14.90°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°N, 14.92°E, Friel et al., August 2002: CUMV 88075, tag no. 5075, 71.0 mm SL, sex undetermined; CUMV 88081, tag no. 5084, 73.0 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°N, 15.12°E, Friel et al., August 2002: CUMV 88125, tag no. 5396, 74.0 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°N, 14.92°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°N, 14.93°E, Friel et al., August 2002: CUMV 88067, tag no. 5029, 59.0 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°N, 15.11°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°N, 24.24°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°N, 24.24°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 ≥ 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 ≤ 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 ≥ 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 mm). Body ovoid, longer than high $(2.3 \le SL/H \le 2.8, average =$ 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 ($6.5 \le HL/SNL \le 9.3$, average = 7.5, holotype = 8.3) and round. Eye large $(3.5 \le \text{HL/ED} \le 4.4, \text{ average} = 4.0, \text{ average}$ holotype = 4.1). Mouth small $(4.1 \le \text{HL/MW} \le 5.0, \text{ average} = 4.6, \text{ holotype} = 4.5),$ 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 $(1.4 \le \text{SL/PDD} \le 1.6)$ and $1.6 \leq SL/PAD \leq 1.7$, respectively). Pre-dorsal distance slightly greater than the preanal distance (PDD/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 $(1.8 \le CPL/CPD \le 2.5, average = 2.2, holotype =$ 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 Odzala-Kokua 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 msec in sexually mature males and 0.270 \pm 0.033 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 msec, duration of peak 2 = 0.075 msec, total EOD duration = 0.260 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-

Petrocephalus binotatus PELLEGRIN, 1924 Holotypus MRAC 15191 (3546) Tetrocephalus binotatus Tell. DET. Telleguin 1914 Bype LOC. Ykengo

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. binotatus*) 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 ≥ 14.7, range = 14.7–18.4 versus 14.2 in *P. boboto* sp. n.), a slightly larger interorbital distance (HL/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 species-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.

_	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
4	caudal mark
_	Eye large (HL/ED \leq 3.5); mouth subterminal but positioned more posteri- orly along the ventral margin of the head (HL/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, ap- pearing to be reversed in polarity compared to EODs of all other <i>Petrocephalus</i> species (see Fig. 11C in Lavoué et al. 2010) Petrocephalus pulsivertens
5	Anal fin contains 30 or more branched rays; melanin markings (black patches) present on the body and always distinctly visible
_	Anal fin contains at most 29 branched rays (usually fewer); melanin markings present but sometimes hardly visible
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
-	Dorsal fin contains 24 or fewer branched rays; eye smaller in size $(3.5 \le \text{HL}/\text{ED} \le 4.7)$; mouth larger (HL/MW ≤ 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	Augenrosette present but reduced in size to few Knollenorgan pores; narrow head (HL/HW = 2.4 and HL/IOW = 3.7) <i>Petrocephalus boboto</i> sp. n.
_	Augenrosette present and well distinct; wider head (HL/HW ≤ 2.2 and HL/ IOW ≤ 3.9 , average = 3.2)
8	Large mouth (SL/MW = 11.6 and HL/MW = 3.2); interorbital distance large (HL/IOW = 2.3); 24 dorsal fin branched rays <i>Petrocephalus binotatus</i>

Smaller mouth (SL/MW \geq 14.7 and HL/MW \geq 4.1); shorter interorbital distance (HL/IOW \ge 2.6); 22 or fewer dorsal fin branched rays..... 9 Melanin markings on body intensely black with sharply defined edges, forming characteristic shapes (e.g., very rounded black sub-dorsal spot or saddlelike sub-dorsal patch, crescent shaped black mark at the base of the caudal fin or round black spot at the caudal fin base)10 Melanin markings of much weaker intensity, consisting of more irregularlyshaped patches and with diffuse edges.....12 10 Small but intense black mark present on each side of the body at the pectoral fin origin; Knollenorgan electroreceptors on the head may or may not be arranged into discrete clusters (i.e., rosettes may be present or absent), but if present, Augenrosette is always as well developed as other two rosettes11 No distinct black mark visible at the origin of the pectoral fin; electroreceptors organized into three distinct rosettes on the head, but the Augenrosette is small and not as well developed as other two rosettes Petrocephalus christyi Eye large (HL/ED \leq 3.3); sub-dorsal black patch often contacting contralat-11 eral mark over dorsum and anteriormost branched rays of the dorsal fin; caudal melanin mark forming a rather uniformly shaped crescent (or "V") extending onto upper and lower fleshy lobes of caudal fin; Knollenorgan electroreceptors on the head not clustered into discrete groups (i.e., rosettes Eye small (HL/ED \ge 3.7); sub-dorsal black patch distinctly rounded, never in contact with contralateral mark and not extending onto dorsal fin; caudal mark ovoid rather than crescent- or V-shaped, not extending onto upper and lower parts of caudal fin; Knollenorgans on the head clustered into three rosettes......Petrocephalus odzalaensis 12 Mouth subterminal, opening under the anterior half of the eye; snout short (HL/SNL \ge 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, P3, 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

Acknowledgments

Specimens were collected and exported under permits issued by the Congolese Fisheries Authority (Direction des Pêches) and the University of Kisangani and were handled in accordance with a protocol for fishes approved by the Cornell University Institutional Animal Care and Use Committee. We thank J. Friel and C. Dardia (Cornell University Museum of Vertebrates, Ithaca, NY, USA) as well as O. Crimmen and J. Maclaine (Natural History Museum, London) for access to the collections under their care. A. Kankonda, Director of the Laboratoire d'Hydrobiologie et Aquaculture (LHA) of the Science Faculty, University of Kisangani provided logistical support and lab space. J.-P. Thumitho of the LHA provided assistance with fieldwork. Comments from J. Friel, Zookeys editor C. Baldwin and two anonymous reviewers improved a previous version of this manuscript. S. L. received support from a postdoctoral fellowship from the National Science Council of Taiwan (NSC102-2811-M-002-071) and travel to Democratic Republic of Congo was provided by NSF grant IOS 0818305 to Carl D. Hopkins. A 2010 U.S. State Department Fulbright Research Scholarship ("Upper Congo Fishes Project") and an Encyclopedia of Life Rubenstein Fellowship supported J.P.S.

References

- Arnegard ME, Bogdanowicz SM, Hopkins CD (2005) Multiple cases of striking genetic similarity between alternate electric fish signal morphs in sympatry. Evolution 59: 324–343. doi: 10.1111/j.0014-3820.2005.tb00993.x
- Arnegard ME, Carlson BA (2005) Electric organ discharge patterns during group hunting by a mormyrid fish. Proceedings of the Royal Society B-Biological Sciences 272: 1305–1314. doi: 10.1098/rspb.2005.3101
- Arnegard ME, McIntyre PB, Harmon LJ, Zelditch ML, Crampton WGR, Davis JK, Sullivan JP, Lavoué S, Hopkins CD (2010a) Sexual signal evolution outpaces ecological divergence during electric fish species radiation. The American Naturalist 176(3): 335–356. doi: 10.1086/655221
- Arnegard ME, Zwickl DJ, Lu Y, Zakon HH (2010b) Old gene duplication facilitates origin and diversification of a new communication system—twice. Proceedings of National Academy of Sciences, USA 107: 22172–22177. doi: 10.1073/pnas.1011803107
- Bigorne R, Paugy D (1991) Note sur la systématique des *Petrocephalus* (Teleostei, Mormyridae) d'Afrique de l'Ouest. Ichthyological Exploration of Freshwaters 2: 1–30.
- Boden G, Teugels GG, Hopkins CD (1997) A systematic revision of the large-scaled *Marcusenius* with description of a new species from Cameroon (Teleostei; Osteoglossomorpha; Mormyridae). Journal of Natural History 31: 1645–1682. doi: 10.1080/00222939700770881
- Bullock TH, Hopkins CD, Popper AN, Fay RR (2005) Electroreception. Springer Science+Business Media, Inc., New York, 472 pp.
- Carlson BA, Arnegard ME (2011) Neural innovations and the diversification of African weakly electric fishes. Communicative & Integrative Biology 4: 1–6. doi: 10.4161/cib.4.6.17483

- Carlson BA, Hasan SM, Hollmann M, Miller DB, Harmon LJ, Arnegard ME (2011) Brain evolution triggers increased diversification of electric fishes. Science 332: 583–586. doi: 10.1126/science.1201524
- Daget J (2000) Les poissons dans l'œuvre du Dr. A.T. de Rochebrune (1833-1912). Cybium 24: 273–286.
- Eschmeyer WN, Fong JD (2014) Species by family/subfamily. Catalog of Fishes electronic version accessed March 2014 http://research.calacademy.org/research/ichthyology/catalog/ SpeciesByFamily.asp
- Harder W (1968) Zum Aufbau der epidermalen Sinnesorgane der Mormyridae (Mormyriformes, Teleostei). Zeitschrift für Zellforschung 89: 212–224. doi: 10.1007/BF00347294
- Harder W (2000) Mormyridae and other Osteoglossomorpha. CD-Rom ETI.
- Kramer B, Bills R, Skelton P, Wink M (2012) A critical revision of the churchill snoutfish, genus *Petrocephalus* Marcusen, 1854 (Actinopterygii: Teleostei: Mormyridae), from southern and eastern Africa, with the recognition of *Petrocephalus tanensis*, and the description of five new species. Journal of Natural History 46: 2179–2258. doi: 10.1080/00222933.2012.708452
- Lavoué S (2011) A new species of *Petrocephalus* Marcusen 1854 (Osteoglossomorpha: Mormyridae) from the Sanaga River basin, Cameroon. Zootaxa 2934: 20–28.
- Lavoué S (2012) *Petrocephalus* Marcusen, 1854 (Osteoglossomorpha: Mormyridae) of the Bangweulu-Mweru ecoregion (Luapula River system, Congo basin), with the description of a new species. Journal of Natural History 46: 2159–2178. doi: 10.1080/00222933.2012.708449
- Lavoué S, Arnegard ME, Sullivan JP, Hopkins CD (2008) *Petrocephalus* of Odzala offer insights into evolutionary patterns of signal diversification in the Mormyridae, a family of weakly electrogenic fishes from Africa. Journal of Physiology-Paris 102: 322–339. doi: 10.1016/j. jphysparis.2008.10.003
- Lavoué S, Hopkins CD, Kamdem Toham A (2004) The *Petrocephalus* (Pisces, Osteoglossomorpha, Mormyridae) of Gabon, Central Africa, with the description of a new species. Zoosystema 26: 511–535.
- Lavoué S, Sullivan JP, Arnegard ME (2010) African weakly electric fishes of the genus *Petrocephalus* (Osteoglossomorpha: Mormyridae) of Odzala National Park, Republic of the Congo (Lekoli River, Congo River basin) with description of five new species. Zootaxa 2600: 1–52.
- Moller P (1995) Electric fishes: History and behavior. Chapman and Hall, London, 584pp.
- Pellegrin J (1924) Description de Mormyridés nouveaux récoltés au Congo belge par le Dr. Schouteden. Revue de Zoologie Africaine 12: 1–8.
- Pellegrin J (1928) Poissons du Chiloango et du Congo recueillis par l'expédition du Dr. H. Schouteden (1920-22). Annales du Musée du Congo, Zoologie 3: 1–50.
- Pezzanite B, Moller P (1998) A sexually dimorphic basal anal-fin ray expansion in the weakly discharging electric fish *Gnathonemus petersii*. Journal of Fish Biology 53: 638–644.
- Poll M, Gosse JP (1963) Contribution à l'étude systématique de la faune ichthyologique du Congo Central. Annales du Musée Royal de l'Afrique Centrale, Sciences Zoologiques 116: 43–111.
- Rabosky DL, Santini, F, Eastman J, Smith SA, Sidlauskas B, Chang J, Alfaro ME (2013) Rates of speciation and morphological evolution are correlated across the largest vertebrate radiation. Nature Communications 4: 1–8. doi: 10.1038/ncomms2958

- Silvestro D, Michalak I (2012) raxmlGUI: a graphical front-end for RAxML. Organisms Diversity & Evolution 12: 335–337. doi: 10.1007/s13127-011-0056-0
- Stamatakis A (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22: 2688–2690. doi: 10.1093/bioinformatics/btl446
- Sullivan JP, Lavoué S, Hopkins CD (2000) Molecular systematics of the African electric fishes (Mormyroidea: Teleostei) and a model for the evolution of their electric organs. Journal of Experimental Biology 203: 665–683.
- Taverne L (1969) Etude ostéologique des genres Boulengeromyrus Taverne et Géry, Genyomyrus Boulenger, Petrocephalus Marcusen (Pisces Mormyriformes). Annales du Musée Royal de l'Afrique Centrale, Sciences Zoologiques 174: 1–85.
- Taverne L (1972) Ostéologie des genres Mormyrus Linné, Mormyrops Müller, Hyperopisus Gill, Myomyrus Boulenger, Stomatorhinus Boulenger et Gymnarchus Cuvier. Considérations générales sur la systématique des poissons de l'ordre des Mormyriformes. Annales du Musée Royal de l'Afrique Centrale, Sciences Zoologiques 200: 1–194.
- Turner RW, Maler L, Burrows M (1999) Electroreception and electrocommunication. Journal of Experimental Biology 202: 1167–1458.

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).
- Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: doi: 10.3897/zookeys.400.6743.app1

RESEARCH ARTICLE



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

Rainer Neumeyer^{1,†}, Hannes Baur^{2,‡}, Gaston-Denis Guex^{3,§}, Christophe Praz^{4,|}

I Probsteistrasse 89, CH-8051 Zürich, Switzerland **2** Abteilung Wirbellose Tiere, Naturhistorisches Museum der Burgergemeinde Bern, Bernastrasse 15, CH-3005 Bern, Switzerland **3** Institute of Evolutionary Biology and Environmental Studies, Field Station Dätwil, University of Zurich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland **4** Evolutionary Entomology, Institute of Biology, University of Neuchatel, Emile-Argand 11, CH-2000 Neuchâtel, Switzerland

thttp://zoobank.org/B0BFC898-8FDF-4E01-8657-CBADE08292D3
 http://zoobank.org/76BB8FCD-3EC9-4774-8FF3-194D57A8A905
 http://zoobank.org/D038CAA8-4662-4BD9-931A-C0FD5FC623D8
 http://zoobank.org/0718546C-5D1A-44D4-921C-C7EA0C11BE0A

Corresponding author: Rainer Neumeyer (neumeyer.funk@bluewin.ch)

Academic editor: M. Buffington | Received 13 November 2013 | Accepted 18 March 2014 | Published 11 April 2014

http://zoobank.org/91DC4784-F49A-4353-B4C8-DC0F67B1EF92

Citation: Neumeyer R, Baur H, Guex G-D, Praz C (2014) A new species of the paper wasp genus *Polistes* (Hymenoptera, Vespidae, Polistinae) in Europe revealed by morphometrics and molecular analyses. ZooKeys 400: 67–118. doi: 10.3897/ zookeys.400.6611

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. bischoffi* 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*.

Copyright Rainer Neumeyer et al. This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

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. bischoffi*; we review the information on the type material of *bischoffi*, and designate a neotype to settle the status of this species. *P. bischoffi* 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, Dvořák 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 bischoffi* (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 bischoffi* 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 phenol-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. bischoffi* 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 °C, then six cycles of 1 min at 94 °C, 1.5 min at 45 °C, and 1.25 min at 72 °C, followed by 36 cycles of 1 min at 94 °C, 1.5 min at 51 °C, and 1.25 min at 72 °C, with a final step of 5 min at 72 °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 °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

Unit name	Voucher No	COX1	ITS1	Locality
		Outgrou	p	
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
	1	Ingroup		1
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 bischoffi	22	KJ415838	KJ415938	CH, Pfäffikon
Polistes bischoffi	75	KJ415839	KJ415939	CH, Wetzikon
Polistes bischoffi	76	KJ415840	KJ415940	CH, Wetzikon
Polistes bischoffi	77	KJ415841	-	CH, Wetzikon
Polistes bischoffi	82	KJ415842	KJ415941	CH, Wetzikon
Polistes bischoffi	83	-	KJ415942	CH, Wetzikon
Polistes bischoffi	105	KJ415843	KJ415943	CH, Wetzikon
Polistes bischoffi	135	KJ415844	KJ415944	CH, Greifensee
Polistes bischoffi	136	KJ415845	KJ415945	CH, Greifensee
Polistes bischoffi	137	KJ415846	KJ415946	CH, Mönchaltorf
Polistes bischoffi	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

 Table 1. Locality information, voucher numbers and GenBank accession numbers for sequences used in this study.

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

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

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

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 δ .

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.

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	Městské muzeum Mariánské Lázně, 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
СМ	Christian Monnerat
CSE	Christian Schmid-Egger
ES	Erwin Steinmann
FA	Felix Amiet
GA	Georg Artmann-Graf
HT	Hansueli Tinner
IK	Igor Kramer

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

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. bischoffi* represent two distinct, well-supported clades (Bootstrap support, hereafter BS, of 100 and 94%, respectively). Sequences of all included specimens of *P. bischoffi*, 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. hellenicus* and for *P. gallicus*, 1.06% for *P. sp. aff. gallicus*, and 1.4% for



Figure 1. 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. dominu-la* 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

79



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. bischoffi*, 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. hellenicus*.

The smallest interspecific distance in ITS1 sequences was 0.8%, between *P. bi-glumis* and *P. hellenicus*; the maximal distance in our ingroup was 11.5%, observed between *P. nimpha* and *P. biglumis*. The minimum distance between *P. bischoffi* 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. bischoff 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.

biglumis									
	females n=19 males 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.l	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.l	1580	1920	1751.6	94.83	1540	1840	1695	59.78	
msp.l	371	514	445.5	34.85	371	436	403.6	22.53	
ool.l	514	629	578.6	31.59	471	571	529.3	29.03	
pol.l	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.l	2900	3767	3387.7	250.73	3000	3733	3428.3	163.04	
bischoffi									
		female	s n=46		males 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.l	671	886	781.1	53.03	871	1000	944	30.56	
flglast.b	257	314	286.5	14.85	150	171	158.2	7.05	
flglast.l	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.l	1340	1720	1516.1	102.99	1460	1620	1520	48.99	
msp.l	250	350	289.4	22.57	171	207	187.4	10.97	
ool.l	429	529	472.8	26.92	379	479	417	28.35	
pol.l	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.l	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.l	2600	3633	3072.5	256.69	2767	3233	3061.5	156.26	
gallicus									
	females n=42			males 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.l	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.l	286	357	313.6	18.92	329	457	385.4	33.02	

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

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	
				hellenicus				·	
		female	s n=21		males 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.l	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.l	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					
		female	s 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.l	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 b males c, d Scatterplot of isosize against first shape PC c females d males. Symbols: orange circles = *biglumis*, purple triangles = *bischoffi*, 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. bischoffi* 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 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 bischoffi* with *P. helveticus*: **a** females **b** males. Symbols: purple triangles = *bischoffi*, 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.



Figure 5. PCA ratio spectrum (blue) and allometry ratio spectrum (green) of a comparison of *Polistes bischoffi* with *P. helveticus*. **a** females **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 :

Polistes bischoffi-helveticus, females

Polistes bischoffi-helveticus, males

females									
group comparison	best ratio	range group 1	range group 2	standard distance	δ				
<i>biglumis</i> _rest*	msp.l : pol.l	1.22–1.76	0.79–1.24	4.62	0.29				
helveticus–bischoffi*	tib3.l : msp.l	7.61–9.33	9.57-11.5	6.19	0.10				
helveticus–gallicus	hea.b : msp.l	7.29-8.80	8.23-10.08	4.34	0.05				
helveticus–hellenicus	tib3.l : msp.l	7.61–9.33	9.00-11.13	4.72	0.09				
bischoffi–gallicus	flglast.l : msp.l	1.00-1.24	0.84-1.07	4.07	0.19				
bischoffi–hellenicus	flglast.l : msp.l	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	δ				
<i>biglumis</i> _rest*	lof.l : cly.b	1.29–1.45	1.46–1.69	8.36	0.31				
helveticus–bischoffi*	flglast.l : msp.l	1.08-1.72	1.93-2.75	6.82	0.08				
helveticus–gallicus	flglast.l : pol.l	0.83-1.32	1.09-1.55	3.54	0.09				
helveticus–hellenicus	flglast.l : msp.l	1.08-1.72	1.44-2.16	5.92	0.01				
bischoffi–gallicus	flglast.l : msp.l	1.93–2.75	1.28-2.21	3.50	0.18				
bischoffi—hellenicus*	hea.b : hea.h	1.21–1.29	1.15-1.21	6.27	0.10				

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.

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, δ (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 6.96–8.56; 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. bischoffi*.

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* sp. 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 \mathcal{J} (Lectotype of *P. dubius*): AUSTRIA, LOWER AUS-TRIA, **Brühl**, 22 Aug 1883, Franz Friedrich Kohl det., NHMW coll.; $2 \stackrel{?}{\lhd}$ (RN0123, RN0124): CROATIA, ISTRIA, Vela Učka, 45°18'25.7"N, 14°11'40.4"E, 824 m, 27 Jul 2012, karst mountain range, Rainer Neumeyer leg., RN coll.; 1 👌 (RN0224): GERMANY, BADEN-WÜRTTEMBERG, Pullendorf, 13 Jul 2009, railroad area, Mike Herrmann leg., MH coll.; 1 \bigcirc (Holotype of *bimaculatus nigrinotum*: ZSM-HYM-000008): BAVARIA, Althegnenberg, 19-20 Jul 1946, Heinz Freude leg., ZSM coll.; 1 3 (RN0231): SWITZERLAND, CANTON BASLE-CITY, Basel, Badischer Bahnhof, 47°34'50.12"N, 07°36'07.63"E, 255 m, 18 Aug 1995, railroad area, Rainer Neumeyer leg., RN coll.; 1 👌 (RN0247): CANTON GRISONS, Buseno, Monti di San Carlo, 1200 m, 09 Jul 1942, Adolf Nadig leg., ETHZ coll.; 1 ♀ (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): Klosters-Serneus, Boschga, 46°52'52.20"N, 09°52'14.34"E, 1060 m, 01 May 1993, montane meadow, Rainer Neumeyer leg., RN coll.; 2 3 (RN0250, RN0258): Ramosch, 07 Sep 1963, Willi Sauter leg., ETHZ coll.; 1 ♀ (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°37'02.21"N, 10°25'56.36"E, 1420 m, 13 Aug 2011, rock steppe, Rainer Neumeyer leg., RN coll.; 1 d (RN0027): Val Müstair, Sta. Maria: Costas, 46°36'25.78"N, $10^{\circ}25'29.37''E$, 1350 m, 13 Aug 2011, berm, Rainer Neumeyer leg., RN coll.; 1 \bigcirc (RN0029): Val Müstair, Tschierv: God da Munt, 46°37'48.79"N, 10°20'42.11"E, 1790 m, 14 Aug 2011, clear larch wood, Rainer Neumeyer leg., RN coll.; 1 \bigcirc + 1 \checkmark (RN0021, RN0087): Hansueli Tinner leg., RN coll.; 1 ♀ (RN0243): **Zuoz**, 1800 m, 09 Sep 1966, Paul Bovey leg., ETHZ coll.; 1 👌 (RN0233): CANTON ST. GAL-LEN, Pfäfers, Bläser Berg, 46°57'24.76"N, 09°29'51.11"E, 1500 m, 23 Aug 1994, blowdown, Peter Duelli leg., RN coll.; 1 d (RN0256): Pfäfers, 20 Sep 1955, Hedwig Huber leg., ETHZ coll.; 1 👌 (RN0254): Walenstadt, Steinbruch Engen, 04 Sep 1997, quarry, Andreas Müller leg., ETHZ coll.; 1 👌 (RN0232): CANTON SCHAFF-HAUSEN, Merishausen, Chörblihalde, 47°45'24.82"N, 08°37'16.59"E, 565 m, 25 Aug 2004, hay meadow, Rainer Neumeyer leg., RN coll.; 1 Q (RN0228): Merishausen, Laadel, 47°46'17.63"N, 08°36'25.42"E, 620 m, 16 Aug 2004, fallow meadow, Rainer Neumeyer leg., RN coll.; 1 ♀ (RN0225): CANTON THURGOVIA, Herdern, 47°36'39.59"N, 08°54'20.39"E, 635 m, Aug 2005, Mike Herrmann leg., MH coll.; 1 Q (RN0240): CANTON TICINO, Airolo, 04 Aug 1933, Adolf Nadig leg., ETHZ coll.; 1 ♀ (RN0227): Lavertezzo, Piano, 46°15′54.34″N, 08°49′15.23″E, 589 m, 01 Jun 2012, Yannick Chittaro leg., YC coll.; 1 d (RN0236): Prato Sornico, Lovalt, 46°23'20.36"N, 08°39'51.38"E, 610 m, 24 Jul 1994, riparian zones, Rainer Neumeyer leg., RN coll.; 1 Q (RN0178): Prugiasco, San Carlo di Negrentino, 46°27'46.14"N, 08°55'24.50"E, 860 m, 20 Aug 1993, montane meadow, Rainer Neumeyer leg., RN coll.; 1 Q (RN0257): CANTON VALAIS, Ausserberg, Millachra,

46°19'08.04"N, 07°50'14.02"E, 1210 m, 09 Jul 1998, Rainer Neumeyer leg., RN coll.; 1 \bigcirc (RN0234): 06 Sep 1998, Rainer Neumeyer leg., RN coll.; 1 \bigcirc (RN0244): **Erschmatt, Rüemetschbodu**, 46°19'35.42"N, 07°41'44.68"E, 1490 m, 18 Jul 2003, Alexandra Breitenstein leg., ETHZ coll.; 1 \bigcirc (RN0252): **Fiesch**, 28 Jul 1942, Adolf Nadig leg., ETHZ coll.; 1 \bigcirc (RN0253): **Grimentz**, 18 Aug 1941, Adolf Nadig leg., ETHZ coll.; 1 \bigcirc (RN0235): **Guttet-Feschel**, 1300 m, 1993, Gerhard Bächli leg., RN coll.; 1 \bigcirc (RN0241): **Ried-Brig, Berisal**, 30 Jun 1919, anon. leg., ETHZ coll.; 1 \bigcirc (RN0226): **Ried-Brig, Gantertal**, 46°17'56.61"N, 08°03'35.70"E, 1420 m, 26 Jun 2012, Yannick Chittaro leg., YC coll.; 3 \bigcirc (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. bischoffi, 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 bischoff). 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. bischoffi (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 "*bischoffi*", 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 **b** lateral view of lower face with malar space and mandible **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 bischoffi*. 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 bischoffi. 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. bischoffi) that appears at the



Figure 7. Different aspects of a male (specimen RN0151) of *Polistes bischoffi* Weyrauch, 1937: **a** frontal view of head **b** lateral view of lower face with malar space and mandible **c** dorsal view of body **d** lateral view of head, mesosoma, and base of metasoma **e** lateral view of body. The red arrow in **b** is pointing to the faint lateral ridge on clypeus. In **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. 6e; 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 mm (n = 22); forewing length 7.8-11.4 mm (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 mm (n = 8); forewing length 9.3-9.8 mm (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. bischoffi, P. helveticus* sp. 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 \bigcirc (RN0366): FRANCE, CORSICA, Galeria, 42°25'11"N, 08°39'37"E, 0 m, 19 Apr 2002, estuary, Christian Monnerat leg., NMBE coll.

Paralectotype: 1 \bigcirc (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!] \bigcirc gallicus L." [handwritten, possibly from v. Leonhardi; according to Stephan Blank (pers. comm.)]; 4. "Weyrauch det. 1937." [handwritten]; 5. "Polistula bischoffi Weyrauch" [handwritten; possibly from Weyrauch; according to Stephan Blank (pers. comm.)]".

Further material: 1 \bigcirc (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 \bigcirc (RN0323): FRANCE, BOUCHES-DU-RHÔNE, Miramas, Étang de Berre, 15 Jul 1979, M. Kühbandner leg., MSNV coll.; 1 ♀ (RN0380): Saintes-Maries-de-la-Mer, Camargue, 28 Jul 2002, J. & I. Smit leg., JS coll.; 1 ♀ (RN0381): HÉRAULT, Vendres, 43°13'00"N, 03°14'38"E, 0 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 Q (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 \bigcirc (RN0370): VAUCLUSE, **Villelaure**, 18 Jul 2000, Jan Smit leg., JS coll.; 1 \bigcirc (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.; $2 \ \bigcirc$ (RN0392, RN0393): LACONIA, **Chosiari, Vathi**, 09 Jun 1998, beach, Werner Arens leg., WA coll.; 1 (RN0322): ITALY, LAZIO, Roma, Torrimpietra, 10 Aug 1971, Heiss leg., MSNV coll.; 1 \mathcal{Q} (RN0410): **Roma,** Sep 1942, O. Querci, MSNM coll.; 1 \mathcal{Q} (RN0411): LOM-BARDIA, Guardamiglio, Fiume Po, 12 Aug 1974, river bank, Vincenzo Ferri

leg., MSNM coll.; 1 \bigcirc (RN0409): PIEMONTE, Cameri, Cascina Galdina, 11 Jul 1981, glade, Vincenzo Ferri leg., MSNM coll.; 1 Q (RN0371): Lombardore, Sep 1972, Osella leg., MCSNV coll.; 2 ♀ (RN0170, RN0171): SWITZERLAND, CANTON GENEVA, Versoix, "vers la Versoix", 1 Jul 1927, anon. leg., MHNG coll.; 1 ♀ (RN0156): CANTON VAUD, Chabrey, La Morette, 8 Sep 1992, fen, Richard Vernier leg., MHNN coll.; $4 \, \bigcirc \, (RN0135, RN0136, RN0141, RN0148)$: CANTON ZURICH, Greifensee, Böschen: 47°22'21.46"N, 08°40'03.38"E, 436 m, 11 Aug 2010, fen rotation fallow, Rainer Neumeyer leg., MZL coll.; 1 ♀ + 1 ♂ (RN0147, RN0150): 47°22'21.46"N, 08°40'03.38"E, 436 m, 11 Aug 2010, fen rotation fallow, Rainer Neumeyer leg., AMNH coll.; 1 Q (RN0146): 47°22'20.36"N, 08°40'02.49"E, 436 m, 11 Aug 2010, fen rotation fallow, Rainer Neumeyer leg., CM coll.; 1 Q (RN0142): 47°22'20.59"N, 08°40'02.64"E, 436 m, 11 Aug 2010, fen rotation fallow, Rainer Neumeyer leg., MH coll.; $1 \stackrel{\bigcirc}{\downarrow} (RN0137) + 1 \stackrel{\bigcirc}{\bigcirc} (RN0151)$: Mönchaltorf, Seewisen, 47°19'17.08"N, 08°41'56.05"E, 436 m, 11 Aug 2010, fen rotation fallow, Rainer Neumeyer leg., NMBE coll.; $1 \bigcirc (RN0140)$: 47°19'17.80"N, 08°41'54.97"E, 436 m, 11 Aug 2010, fen rotation fallow, Rainer Neumeyer leg., CSE coll.; 1 ♀ (RN0001): **Pfäffikon, Auslikon,** 47°20'47.75"N, 08°47'50.16"E, 539 m, 10 May 2011, fen, Rainer Neumeyer leg., RN coll.; 1 d (RN0022): Pfäffikon, Birchen, 47°21'03.19"N, 08°47'31.73"E, 538 m, 10 Aug 2011, fen, Rainer Neumeyer leg., RN coll.; 1 Q (RN0169): Regensdorf, Altburg, 24 Jul 1997, hill near fen, Bernhard Merz leg., MHNG coll.; 1 Q (RN0145): Schwerzenbach, Böschen, 47°22'21.48"N, 08°40'01.19"E, 436 m, 11 Aug 2010, fen rotation fallow, Rainer Neumeyer leg., NML coll.; 1 ♀ (RN0149): 47°22'21.35"N, 08°40'00.85"E, 436 m, 11 Aug 2010, fen rotation fallow, Rainer Neumeyer leg., NHMB coll.; $1 \stackrel{\frown}{\downarrow} (RN0143)$: 47°22'21.39"N, 08°40'00.75"E, 436 m, 11 Aug 2010, fen rotation fallow, Rainer Neumeyer leg., ETHZ coll.; 1 ♀ (RN0144): 47°22'21.39"N, 08°40'00.90"E, 436 m, 11 Aug 2010, fen rotation fallow, Rainer Neumeyer leg., MCSNL coll.; 1 \heartsuit (RN0076): Wetzikon, Himmerich: 47°20'07.85"N, 08°47'31.05"E, 540 m, 02 Sep 2011, fen, Rainer Neumever leg., RN coll.; 1 Q (RN0105): Wetzikon, Robenhuserriet, 47°20'11.31"N, 08°47'14.84"E, 538 m, 17 Aug 2012, fen, Rainer Neumeyer leg., BNM coll.; 1 (RN0075): Wetzikon, Seeriet, 47°20'30.24"N, 08°47'10.89"E, 538 m, 02 Sep 2011, fen, Rainer Neumeyer leg., RN coll.; 2 🖧 (RN0082, RN0083): 47°20'30.24"N, 08°47'10.89"E, 538 m, 09 Sep 2011, fen, Rainer Neumeyer leg., RN coll.; 1 ♀ (RN0077): 47°20'29.54"N, 08°47'23.73"E, 537 m, 02 Sep 2011, fen, Rainer Neumeyer leg., RN coll.; 1 🖧 (RN0328): Zürich, Katzensee Allmend, 47°25'53.05"N, 08°30'26.15"E, 438 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 👌 (RN0334): 19 Aug 2013, Rainer Neumeyer leg., NHM coll.; $2 \ (RN0337, RN0338) + 2 \ (RN0339, RN0340)$: 47°25'56.74"N, 08°30'22.41"E, 438 m, 19 Aug 2013, fen, Rainer Neumeyer leg., RN coll.; 3 ♀ (RN0363, RN0364, RN0365): TURKEY, HEKARÎ, road from Yüksekova to Semdinli ["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 (Lectotype of *P. foederatus*): AZERBAIJAN, GOYGOL DISTRICT, Göygöl ["Transkauk., Helenendorf"], 1886, Franz Friedrich Kohl det., NHMW coll.; 1 \bigcirc (Holotype of *P. omissus ordubadensis*: ZSM-HYM-000005): NAKHCHIVAN AUTONOMOUS REPUBLIC, Ordubad, 1913, Kulzer leg., ZSM coll.; 1 d (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 Q (RN0099): CROATIA, IS-TRIA, Rovinj, Cesta za Mondelaco, 45°05'57.6"N, 13°38'36.8"E, 15 m, 24 Jul 2012, fallow, Rainer Neumeyer leg., CSE coll.; 1 ♀ (RN0100): 28 Jul 2012, fallow, Rainer Neumeyer leg., CSE coll.; 3 Q (RN0194, RN0195, RN0196): Rainer Neumeyer leg., RN coll.; 1 ♀ (RN0009): Rovinj, Farma Haber, 45°06'40.8"N, 13°40'21.0"E, 30 m, 22 Jul 2011, farm, Rainer Neumeyer leg., RN coll.; 1 Q (RN0197): Vodnjan, street D21, 44°57'58.0"N, 13°50'47.4"E, 133 m, 24 Jul 2012, fallow, Rainer Neumeyer leg., RN coll.; 2 \bigcirc (Syntypes of *P. foederatus obscuricornis*; RN0444, RN0445): KRK, \leq 1936, NHMW coll.; 1 \bigcirc (RN0343): ITALY, SARDINIA, Cabras, Stagno di Cabras, 39°57'07.6"N, 08°31'17.2"E, 5 m, 31 Aug 2013, paddy, Rainer Neumeyer leg., RN coll.; 1 👌 (RN0347): Strada Provinciale 1, 39°55'14.1"N, 08°31'17.4"E, 6 m, 01 Sep 2013, reed, Rainer Neumeyer leg., RN coll.; $1
ot \subseteq (RN0344)$: Macomer, Via Alagon, 40°15'49.9"N, 08°46'54.5"E, 553 m, 01 Sep 2013, rock face, Rainer Neu-

meyer leg., RN coll.; 1 \bigcirc (RN0345) + 1 \bigcirc (RN0348): Scano di Montiferro, Street SP78, 40°13'19.9"N, 08°36'29.9"E, 442 m, 01 Sep 2013, quarry, Rainer Neumeyer leg., NHM coll.; 1 d (RN0346): Tadasuni, Street to San Serafino, 40°05'41.4"N, 08°52'44.5"E, 138 m, 30 Aug 2013, edge community, Rainer Neumeyer leg., RN coll.; 1 \bigcirc (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 Q (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 ♀ (RN0108): POR-TUGAL, ALGARVE, Vila do Bispo, western shore, 37°06'57.2"N, 08°55'37.2"W, 11 m, 04 Apr 2012, dune, Rainer Neumeyer leg., RN coll.; $1 \ \bigcirc$ (RN0395) + 1 \bigcirc (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 ♀ (RN0118): San Vittore, Ai Tecc, 46°14'10.42"N, 09°05'20.18"E, 262 m, 30 Jul 2012, industrial fallow, Rainer Neumeyer leg., RN coll.; 1 \mathcal{J} (RN0166): **Savognin,** Jul 1907, anon. leg., BNM coll.; 1 \mathcal{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°09'19.72"N, 08°58'18.65"E, 215 m, 30 Jul 2012, industrial fallow, Rainer Neumeyer leg., RN coll.; 4 ♀ (RN0103, RN0106, RN0107, RN0109): 08 Aug 2012, industrial fallow, Rainer Neumeyer leg., RN coll.; $2 \Im$ (RN0041, RN0042): CANTON VALAIS, Leuk, Satellitenbodenstation, 46°19'06.66"N, 07°38'36.56"E, 919 m, 22 Aug 2011, tall herbaceous vegetation, Rainer Neumeyer leg., RN coll.; 2 \bigcirc (RN0104, RN0113): 07 Aug 2012, Rainer Neumeyer leg., RN coll.; 1 \bigcirc + 2 d (RN0115, RN0116, RN0117): CANTON VAUD, Villars-sous-Yens, Arborex, 46°30'11.03"N, 06°25'12.11"E, 510 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 flagel-lomere 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. bischoffi*, but clearly outside the range of *P. helveticus* sp. n. (Fig. 3b, c [L]).

Material examined. Holotype ♂ (RN0242): GREECE, ARCADIA, Ano Kotili, peak of Lykaion, 1100–1400 m, 07 Jul 2010, Werner Arens leg., WA coll.

Paratypes: 1 \bigcirc (RN0174): GREECE, ARCADIA, **Andritsena**, 1997, Werner Arens leg., ZSM coll.; 1 \bigcirc (RN0125): **Ano Kotili, peak of Lykaion**, 1100–1400 m, 07 Jul 2010, Werner Arens leg., WA coll.; 1 \bigcirc (RN0179): ARGOLIS, **Drepano**, 06 Jul 2008, Werner Arens leg., WA coll.

Further material: 4 🖉 (RN0220, RN0221, RN0222, RN0223): CROATIA, IS-TRIA, Ližnjan, 44°49'N, 13°59'E, 27 Aug 2005, Christian Schmid-Egger leg., CSE coll.; 2 Q (RN0008, RN0088): **Rovinj**, 45°06'07.1"N, 13°39'08.4"E, 22 m, 22 Jul 2011, Rainer Neumeyer leg., RN coll.; 1 \bigcirc (RN0097): **Rovinj, Cesta za Mondelaco,** 45°05'57.6"N, 13°38'36.8"E, 15 m, 24 Jul 2012, Rainer Neumeyer leg., CSE coll.; 1 \bigcirc (RN0098): 28 Jul 2012, Rainer Neumeyer leg., CSE coll.; 1 \bigcirc (RN0193): Rainer Neumeyer leg., RN coll.; 2 Q (RN0010, RN0011): Rovinj, Cesta za Valaltu-Lim, 45°06'16.8"N, 13°38'28.4"E, 26 m, 26 Jul 2011, Rainer Neumeyer leg., RN coll.; 1 \bigcirc (RN0191): 21 Jul 2012, Rainer Neumeyer leg., RN coll.; 1 \bigcirc (RN0193): **Rovinj**, near Valalta, 45°07'02.1"N, 13°37'54.0"E, 14 m, 21 Jul 2012, Rainer Neumeyer leg., RN coll.; 2 👌 (RN0101, RN0102): Vela Učka, 45°18'25.7"N, 14°11'40.4"E, 824 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 \bigcirc (RN0132): **Mantinea**, 08 Jun 2011, Werner Arens leg., WA coll.; 1 \bigcirc (RN0181): ELIS, **Olympia**, 29 May 2011, Werner Arens leg., WA coll.; $1 \ Q$ (RN0131): 08 Jun 2011, Werner Arens leg., WA coll.; $1 \ominus$ (RN0182): Zacharo, Lake Kaiapha, 30 May 2011, Werner Arens leg., WA coll.; $1 \bigcirc (RN0096)$: **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 ♀ (RN0127): MESSENIA, Avia near Kal**amata**, 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.; $1 \stackrel{\bigcirc}{=} (RN0177)$: 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 1.19– 1.27; 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 **b** lateral view of lower face with malar space and mandible **c** mesopleural region of mesosoma **d** lateral view of body **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 mm (n = 20); forewing length 8.5-11.3 mm (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 10. Different aspects of a male (individual RN0153) of *Polistes helveticus* sp. n.: **a** frontal view of head **b** anterolateral view of lower face **c** lateral view of body **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 mm (n = 12); forewing length 8.9–9.9 mm (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. bischoffi, 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. bischoffi*" 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* sp. n. clearly belongs to *P. helveticus* sp. n. according to molecular and morphological analyses. Ac-



Figure 11. Distribution of examined specimens of *Polistes bischoffi* 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 (**g**) or isolated black spot (**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 (**l**) or immediately narrowing (**m**). Drawings **a**, **b**, **c**, **d**, **f** are courtesy of H. Cigler, **g**, **h**, **i**, **k** of D. Lawniczak, and **e**, **l**, **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 (\emptyset 2–3 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 \bigcirc (RN0139): SWITZERLAND, CANTON ZU-RICH, **Schwerzenbach, Böschen,** 47°22'21.67"N, 08°40'01.43"E, 436 m, 11 Aug 2010, fen rotation fallow, Rainer Neumeyer leg., NMBE coll.

Paratypes: 1 \bigcirc (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 👌 (RN0398): Vlkov nad Lužnicí, 18 Sep 2004, P. Bogusch leg., LD coll.; 1 Q (RN0378): FRANCE, ISÈRE, Le Champ-près-Froges, 200 m, 15 Aug 2003, sand pit, J. & I. Smit leg., JS coll.; 1 ♀ (RN0282) + 1 ♂ (RN0284): GER-MANY, BADEN-WÜRTTEMBERG, Blaustein, Lautertal westl. Weidach, 30 Aug 1988, Volker Mauss leg., RN coll.; 1 ♀ (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°22'55.2"N, 11°25'58.8"E, fen, 31 Jul 1991, Stephan Blank leg., SDEI coll.; 1 👌 (RN0162): PRINCIPALITY OF LIECHTENSTEIN, Ruggell, 47°13'56.98"N, 09°39'43.61"E, 444 m, 27 Aug 1996, dam, Simon Bieri leg., ETHZ coll.; 1 👌 (RN0164): Schaan, 47°11'02.53"N, 09°31'29.11"E, 455 m, 24 Sep 1997, Simon Bieri leg., ETHZ coll.; 1 ♀ (RN0400): SLOVAKIA, TRENČÍN REGION, **Bzince pod Javorinou**, 06 Aug 2004, glade, Libor Dvořák leg., LD coll.; 1 ♀ (RN0230): SWITZERLAND, CAN-TON BASLE-CITY, Basel, Badischer Bahnhof, 47°34'50.12"N, 07°36'07.63"E, 255 m, 18 Aug 1995, ruderal field, Rainer Neumeyer leg., RN coll.; 1 Q (RN0377): CAN-TON BERNE, **Bern**, 22 Jul 1883, Theodor Steck leg., NMBE coll.; 1 Q (RN0374) + 2 ♂ (RN0375, RN0376): Bätterkinden, 24 Aug 1887, Theodor Steck leg., NMBE coll.; 1 👌 (RN0161): Gampelen, Seewald, 16 Aug 1994, Richard Vernier leg., MHNN coll.; 1 👌 (RN0158): CANTON JURA, Damphreux, Les Coeudres, 47°28'23.54"N, 07°06'34.74"E, 430 m, 22 Aug 2003, Christian Monnerat leg., MHNN coll.; 1 🖒 (RN0157): Courroux, Le Quenet, 47°22'46.78"N, 07°21'40.20"E, 510 m, 01 Oct 2004, Christian Monnerat leg., MHNN coll.; 2 🖑 (RN0155, RN0159): Courtemaîche, La Colombière, 47°27'48.87"N, 07°02'59.38"E, 390 m, 22 Aug 2003, Christian

Monnerat leg., MHNN coll.; 1 3 (RN0386): CANTON TICINO, Meride, Fontana, 45°53'44"N, 08°56'46"E, 595 m, 24 Aug 1997, Ladislaus Rezbanvai-Reser leg., NML coll.; 1 👌 (RN0387): Muggio, Muggiasca, 45°54'39"N, 09°01'21"E, 980 m, 16 Aug 1996, Ladislaus Rezbanyai-Reser leg., NML coll.; 1 ♀ (RN0394): CANTON VAL-AIS, 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 9 (RN0360) + 1 ♂ (RN0361): Lausanne, Aug 1942, Jacques de Beaumont leg., MFNB coll.; 3 👌 (RN0200, RN0203, RN0204): Lausanne, Vidy, 02 Aug 1943, Jacques de Beaumont leg., MZL coll.; 1 Q (RN0046): Noville, Longes Rayes, 46°23'21.32"N, 06°53'31.03"E, 273 m, 23 Aug 2011, shrubberies, Rainer Neumeyer leg., RN coll.; 5 🖓 (RN0047, RN0048, RN0049, RN0050, RN0051): 46°23'14.28"N, 06°53'34.66"E, 273 m, 23 Aug 2011, tall herbaceous vegetation, Rainer Neumeyer leg., RN coll.; 1 🖑 (RN0160): Saint-Livres, Les Mossières, 46°32'02.47"N, 06°21'55.82"E, 700 m, 21 Aug 2002, Christian Monnerat leg., MHNN coll.; 2 👌 (RN0201, RN0202): Saint-Sulpice, Jul 1943, Jacques de Beaumont leg., MZL coll.; 1 👌 (RN0114): Villars-sous-Yens, Arborex, 46°30'11.03"N, 06°25'12.11"E, 510 m, 25 Aug 2010, fen, Christian Monnerat leg., MHNN coll.; 2 ♀ (RN0277, RN0278): CANTON ZUG, Hünenberg, Rüssspitz, 47°14'09.40"N, 08°24'39.49"E, 389 m, 10 Jul 2012, fen, Rainer Neumeyer leg., ETHZ coll.; 1 ♀ (RN0279): 47°14'17.60"N, 08°24'27.75"E, 389 m, 20 Aug 2012, fen, Rainer Neumeyer leg., AMNH coll.; 1 Q (RN0275): CANTON ZURICH, Bauma, Fischbach, 47°23'00.66"N, 08°50'48.30"E, 660 m, 04 Jul 2012, abandoned pit, Rainer Neumeyer leg., RN coll.; 2 Q (RN0167, RN0168): 08 Aug 2012, abandoned pit, Rainer Neumeyer leg., CSE coll.; 1 👌 (RN0152): Mönchaltorf, Seewisen, 47°19'17.63"N, 08°41'55.58"E, 436 m, 21 Aug 2010, fen rotation fallow, Rainer Neumeyer leg., AMNH coll.; 1 2 (RN0003): Pfäffikon, Auslikon, 47°20'42.05"N, 08°47'52.78"E, 539 m, 20 Jun 2011, fen, Rainer Neumeyer leg., RN coll.; 3 ♀ (RN0018, RN0019, RN0020): 47°20'46.94"N, 08°47'50.38"E, 539 m, 10 Aug 2011, fen, Rainer Neumeyer leg., RN coll.; 1 ♀ (RN0078): Pfäffikon, Irgenhuserriet, 47°20'59.15"N, 08°47'49.98"E, 539 m, 06 Sep 2011, fen, Rainer Neumeyer leg., RN coll.; 2 Q (RN0033, RN0034): Seegräben, Schlachtmad, 47°20'23.35"N, 08°46'36.56"E, 537 m, 19 Aug 2011, fen, Rainer Neumeyer leg., RN coll.; 1 9 + 2 d (RN0138, RN0153, RN0154): Schwerzenbach, Böschen, 47°22'21.67"N, 08°40'01.43"E, 436 m, same nest as holotype, 11 Aug 2010, fen rotation fallow, Rainer Neumeyer leg., NMBE coll.; 1 \bigcirc (RN0276): Weiach, Rüteren, 47°34'03.21"N, 08°26'44.70"E, 365 m, 02 Apr 2005, gravel pit, Rainer Neumeyer leg., RN coll.; 1 ♀ (RN0035): Wetzikon, Agerstenriet, 47°20'06.75"N, 08°46'57.43"E, 538 m, 19 Aug 2011, fen, Rainer Neumeyer leg., RN coll.; $2 \ \bigcirc$ (RN0012, RN0013): Wetzikon, Seeriet, 47°20'30.24"N, 08°47'10.89"E, 538 m, 05 Aug 2011, fen, Rainer Neumeyer leg., RN coll.; 1 \bigcirc (RN0081): 09 Sep 2011, fen, Rainer Neumeyer leg., RN coll.; 1 ♀ (RN0014): Wetzikon, Robenhuserriet, 47°20'19.16"N, 08°47'02.75"E, 538 m, 05 Aug 2011, fen, Rainer Neumeyer leg., RN coll.; 1 Q (RN0017): 47°20'16.20"N, 08°47'34.35"E, 539 m, 05 Aug 2011, fen, Rainer Neumeyer leg., RN coll.; 1 🖒 (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 *gallicus*group. 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 *dominula*-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.

9	Antenna with 10 flagellomeres.	Metasoma with 6	terga1
3	Antenna with 11 flagellomeres.	Metasoma with 7	terga6

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 mandible.
_	Malar space vellow mandible usually black. If vellow area on mandible then
	always smaller than area on malar space dominula-group
2	Elagellum black on upper side bright vellow to orange on lower side (Fig
2	12c) – Metacoxa black Mesoscutum usually without vellow spot (Fig. 12k)
	Clypeus with central, black spot (Fig. 12h) or more often with black, hori-
	zontal bar (Fig. 12g). Hypopygium entirely black
_	Flagellum bright yellow to orange (Fig. 12d) or faintly darkened on upper
	side, but never black
3	Epicnemial carina distinct, marking an abrupt change in sculpture between
	coarse mesepisternum and smooth epicnemium (Fig. 12a). Malar space : lat-
	eral ocelli distance 1.22–1.76. – Mesoscutum with relatively long pubescence
	(Fig. 12a)
_	Epicnemial carina reduced (Fig. 12b) or absent. Change in sculpture between
	mesepisternum and epicnemium often gradual (Fig. 12b). Malar space : lat-
	eral ocelli distance 0.87–1.19
4	Epicnemial carina reduced (Fig. 12b) or absent. Change in sculpture between
	mesepisternum and epicnemium frequently gradual (Fig. 12b). Terminal flag-
	ellomere 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 yel-
	low spot (Fig. 12k), only occasionally with pair of yellow spots (Fig. 12i).
	Metacoxa frequently black, only occasionally spotted yellow. Hypopygium
	entirely black
-	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

Males:

6	Gena convex in dorsal view (Fig. 12l)7
_	Gena converging in dorsal view (Fig. 12m)8
7	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 spotP. 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
8	Epicnemium and mesosternum black (Fig. 8), seldom with pair of elongate
	yellow spots between pro- and mesocoxa
_	Epicnemium and mesosternum entirely yellow (Fig. 7d)9
9	Flagellum black or blackish dorsally, bright yellow to orange ventrally (Fig.
	12e) 10
_	Flagellum entirely bright yellow to orange (Fig. 12f) or faintly darkened dor-
	sally. – Frontal groove reduced11
10	Frontal groove and lateral clypeal ridges reduced. Terminal flagellomere
	length : terminal flagellomere breadth 1.83–2.78 P. helveticus sp. n.
_	Frontal groove and lateral clypeal ridges very distinct. Terminal flagellomere
	length : terminal flagellomere breadth > 2.5 <i>dominula</i> -group (<i>associus</i>)
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. Ter-
	minal flagellomere length : malar space 1.93–2.75 P. bischoffi

5

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. bischoffi, 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. bischoffi*. 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.

Acknowledgements

James Carpenter (AMNH) kindly examined a pair of *Polistes bischoffi* and repeatedly gave invaluable advice during various stages of this work. Very helpful information was also provided by Cornelis van Achterberg (NBC), Stephan Blank (SDEI), Michael Ohl (MFNB), Emilia Perez (FMLT), Claus Rasmussen (MHNL), and Arturo Roig-Alsina (MACN). Adrian Leuchtmann (ETHZ) shared unpublished preliminary sequences of COX2 of several Polistes species. Christian Monnerat (CSCF) kindly agreed to donate the neotype of *P. bischoffi*. DNA sequencing was funded by the CSCF (www.cscf. ch), SwissBOL (www.swissbol.ch) and the NMBE (www.nmbe.ch). Specimens were loaned by Werner Arens (Bad Hersfeld, D), Gerhard Bächli (ZMUZ), Luca Bartolozzi (MSNUF), Marco Bernasconi (NML), Matthias Borer (MHNN), Yannick Chittaro (CSCF), Libor Dvořák (MMML), Anne Freitag (MZL), Bruno Gereys (Oraison, F), Mike Herrmann (Konstanz, D), Leonardo Latella (MCSNV), Andrew Liston (SDEI), Bernhard Merz (MHNG), Elsa Obrecht (NMBE), Maurizio Pavesi (MSNM), Jignasha Rana (HUMCZ), Viola Richter (MFNB), Fabrizio Rigato (MSNM), Wolfgang Schläfle (Kaiseraugst, CH), Franziska Schmid (ETHZ), Christian Schmid-Egger (Berlin, D), Marion Schmid (BNM), Stefan Schmidt (ZSM), Jan Smit (Duiven, NL), Marco Uliana (MSNV), Manuela Vizek (NHMW). Diana Lawniczak (Steffisburg, CH) and Harald Cigler (Affoltern am Albis, CH) contributed drawings to Fig. 12. André Rey designed the map of Fig. 11 and found a nest of *P. bischoffi* in Zurich. Lastly, we thank Matthias Buck and James Carpenter for useful comments on the manuscript, and Jessica Litman for proofreading the final version of the manuscript and for improving the English.

References

- Arens W (2011) Die sozialen Faltenwespen der Peloponnes, mit Beschreibung einer neuen Polistes-Art und einem regionalen Polistes-Bestimmungsschlüssel. Linzer biologische Beiträge 43(1): 443–481.
- Arthofer W, Avtzis DN, Riegler M, Stauffer C (2010) Mitochondrial phylogenies in the light of pseudogenes and *Wolbachia*: re-assessment of a bark beetle dataset. Zookeys 56: 269– 280. doi: 10.3897/zookeys.56.531

- Avtzis DN, Arthofer W, Stauffer C (2008) Sympatric occurrence of diverged mtDNA lineages of *Pityogenes chalcographus* (Coleoptera, Scolytinae) in Europe. Biological Journal of the Linnean Society 94: 331–340. doi: 10.1111/j.1095-8312.2008.01004.x
- Bartels PJ, Nelson DR, Exline RP (2011) Allometry and the removal of body size effects in the morphometric analysis of tardigrades. Journal of Zoological Systematics and Evolutionary Research 49: 17–25. doi: 10.1111/j.1439-0469.2010.00593.x
- Baugnée JY (1996) Une nouvelle guêpe sociale pour la faune belge: *Polistes bischoffi* Weyrauch, 1937, trouvé en Gaume. Bulletin et Annales de la Société Royale Belge d'Entomologie 132: 395–398.
- Baur H, Leuenberger C (2011) Analysis of ratios in multivariate morphometry. Systematic Biology 60: 813–825. doi: 10.1093/sysbio/syr061
- Bergsten J, Bilton DT, Fujisawa T, Elliott M, Monaghan MT, Balke M, Hendrich L, Geijer J, Herrmann J, Foster GN, Ribera I, Nilsson AN, Barraclough TG, Vogler AP (2012) The effect of geographical scale of sampling on DNA barcoding. Systematic Biology 61: 851–869. doi: 10.1093/sysbio/sys037
- Blüthgen P (1943) Die europäischen Polistinen. Archiv für Naturgeschichte, neue Folge 12 (1): 94–129.
- Blüthgen P (1956) Über einige *Polistes*-Arten der Zoolog. Staatssammlung in München. Nachrichtenblatt der Bayerischen Entomologen 5(9): 81–86.
- Blüthgen P (1961) Die Faltenwespen Mitteleuropas. Abhandlungen der Deutschen Akademie der Wissenschaften zu Berlin, Klasse für Chemie, Geologie und Biologie 1961 (2): 251 pp.
- Buck M, Cobb TP, Stahlhut JK, Hanner RH (2012) Unravelling cryptic species diversity in eastern Nearctic paper wasps, *Polistes (Fuscopolistes)*, using male genitalia, morphometrics and DNA barcoding, with descriptions of two new species. Zootaxa 3502: 1–48.
- Buysson MR du (1911) Mission Pelliot-Vaillant dans l'Asie centrale; collections recueillis par M. le Dr. Vaillant, Hyménoptères. Bulletin du Muséum National d'Histoire Naturelle 76 (4): 217–219.
- Carpenter JM (1990) Of genetic distances and social wasps. Systematic Zoology 39(4): 391–397. doi: 10.2307/2992359
- Carpenter JM (1996a) Phylogeny and biogeography of Polistes. In: Turillazzi S, West-Eberhard MJ (Eds) Natural history and evolution of paper wasps. Oxford University Press, Oxford, 18–57.
- Carpenter JM (1996b) Distributional checklist of species of the genus *Polistes*. American Museum Novitates 3188: 1–39.
- Carpenter JM (1997) Phylogenetic relationships among European *Polistes* and the evolution of social parasitism. In: Grandcolas P (Ed) The origin of biodiversity in insects; phylogenetic tests of evolutionary scenarios. Mémoires du Muséum national d'Histoire naturelle, Paris 173: 135–161.
- Castro L, Dvořák L (2009) New and noteworthy records of vespid wasps from the Palaearctic region (II). Boletín Sociedad Entomológica Aragonesa 44: 295–304.
- Cervo R (2006) *Polistes* wasps and their social parasites: an overview. Annales Zoologici Fennici 43: 531–549.
- Cervo R, Dapporto L, Beani L, Strassmann JE, Turillazzi S (2008) On status badges and quality signals in the paper wasp *Polistes dominulus*: body size, facial colour patterns and

hierarchical rank. Proceedings of the Royal Society of London, B 275: 1189–1196. doi: 10.1098/rspb.2007.1779

- Choudhary M, Strassmann JE, Queller DC, Turillazzi S, Cervo R (1994) Social parasites in polistine wasps are monophyletic; implications for sympatric speciation. Proceedings of the Royal Society of London B 257: 31–35. doi: 10.1098/rspb.1994.0090
- Corti C, Masseti M, Delfino M, Pérez-Mellado V (1999) Man and herpetofauna of the Mediterranean islands. Revista Española de Herpetología 13: 83–100.
- Dalla Torre KW von (1904) Vespidae. Genera Insectorum 19: 1–108.
- Day MC (1979) The species of Hymenoptera described by Linnaeus in the genera Sphex, Chrysis, Vespa, Apis and Mutilla. Biological Journal of the Linnean Society 12: 45–84. doi: 10.1111/ j.1095-8312.1979.tb00049.x
- Dvořák L, Roberts SPM (2006) Key to the paper and social wasps of Central Europe. Acta Entomologica Musei Nationalis Pragae 46: 221–244.
- Dvořák L, Smetana V, Straka J, Deván P (2006) Present distribution of the paper wasp *Polistes bischoffi* Weyrauch, 1937 in the Czech Republic and in Slovakia with notes to its spreading. Linzer biologische Beiträge 38(1): 533–539.
- Geoffroy EL (1785) In: Fourcroy AF de (Ed) Entomologia parisiensis, sive catalogus insectorum quae in agro parisiensi reperiuntur, vol. 2: 233–544. Paris.
- Gigon A, Rocker S, Walter T (2010) Praxisorientierte Empfehlungen für die Erhaltung der Insekten- und Pflanzenvielfalt mit Ried-Rotationsbrachen. Forschungsanstalt Agroscope Reckenholz-Tänikon (www.agroscope.ch); ART-Bericht 721: 1–12.
- Giordani Soika A (1970) Ergebnisse der zoologischen Forschungen von Dr. Z. Kaszab in der Mongolei; 223, Vespidae und Eumenidae. Annales historico-naturales Musei Nationalis Hungarici, pars zoologica 62: 325–333.
- Giordani Soika A (1976) Vespidi ed Eumenidi raccolti in Mongolia dal Dr. S. Kaszab. Acta zoologica Academiae Scientiarum Hungaricae 22(3–4): 271–276.
- Gonseth Y, Wohlgemuth T, Sansonnens B, Buttler A (2001) Les regions biogéographiques de la Suisse. Documents environment, 137. Swiss Agency for the Environment, Forests and Landscape, Berne, 48 pp.
- Goulet H, Huber JT (1993) Hymenoptera of the world; an identification guide to families. Research Branch, Agriculture Canada, 668 pp.
- Graf F (1961) Présence de l'Hyménoptère Vespidé *Polistes bischoffi* Weyrauch en France. Bulletin de la Société Zoologique de France 86: 283–291.
- Guiglia D (1967) La posizione del *Polistes (Leptopolistes) bischoffi* Weyrauch. Mitteilungen der Schweizerischen Entomologischen Gesellschaft 39(3+4): 199–204.
- Guiglia D (1972) Les guêpes sociales d'Europe occidentale et septentrionale. Faune de l'Europe et du basin méditerranéen 6: 181 pp.
- Gusenleitner J (1985) Bemerkenswertes über Faltenwespen VIII. Nachrichtenblatt der Bayerischen Entomologen 34: 105–110.
- Gusenleitner J (1995) Hymenopterologische Notizen aus Österreich, 3. Linzer biologische Beiträge 27(1): 159–167.
- Gusenleitner J (1998) Hymenopterologische Notizen aus Österreich, 9. Linzer biologische Beiträge 30(2): 497–501.

- Hebert PDN, Cywinska A, Ball SL, deWaard JR (2003) Biological identifications through DNA barcodes. Proceedings of the Royal Society of London B: Biological Sciences 270: 313–321. doi: 10.1098/rspb.2002.2218
- Hebert PDN, Penton EH, Burns JM, Janzen DH, Hallwachs W (2004) Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. Proceedings of the National Academy of Sciences 101: 14812–14817. doi: 10.1073/ pnas.0406166101
- ICZN (2012) International Code of Zoological Nomenclature. www.nhm.ac.uk/hosted-sites/ iczn/code/
- Janzon LA (1986) Morphometric studies of some *Pteromalus* Swederus species (Hymenoptera: Chalcidoidea) with emphasis on allometric relationships, or: Are ratios reliable in chalcid taxonomy? Systematic Entomology 11: 75–82. doi: 10.1111/j.1365-3113.1986.tb00166.x
- Ji YJ, Zhang DX, He LJ (2003) Evolutionary conservation and versatility of a new set of primers for amplifying the ribosomal internal transcribed spacer regions in insects and other invertebrates. Molecular Ecology Notes 3: 581–585. doi: 10.1046/j.1471-8286.2003.00519.x
- Kenis M, Mills NJ (1998) Evidence for the occurence of sibling species in *Eubazus* spp. (Hymenoptera: Braconidae), parasitoids of *Pissodes* spp. weevils (Coleoptera: Curculionidae). Bulletin of Entomological Research 88: 149–163. doi: 10.1017/S0007485300025724
- Kofler A (2005) Neue Mitteilungen über Keulenwespen, Rollwespen und Echte Wespen aus Osttirol (Österreich). Berichte des Naturwissenschaftlich Medizinischen Vereins in Innsbruck 92: 141–160.
- Kohl FF (1898) Zur Kenntniss [sic!] der europäischen Polistes-Arten. Annalen des kaiserlichköniglichen Naturhistorischen Hofmuseums, Wien 13: 87–90.
- Kwet A (2005) Reptilien und Amphibien Europas. Franckh-Kosmos, Stuttgart, 252 pp.
- László Z, Baur H, Tóthmérész B (2013) Multivariate ratio analysis reveals *Trigonoderus pedicellaris* Thomson (Hymenoptera, Chalcidoidea, Pteromalidae) as a valid species. Systematic Entomology 38: 753–762. doi: 10.1111/syen.12026
- Lepeletier de Saint-Fargeau A, Serville A (1825) [Articles] In: Latreille M (Ed) Encyclopédie Méthodique, Histoire Naturelle. Insectes. Vol. 10. Agasse, Paris, 344 pp.
- Linnaeus C (1758) Systema naturae 1 (Editio decima). Salvius, Holmiae, Stockholm.
- Linnaeus C (1767) Systema naturae 1 (2) (Editio duodecima), 533–1068. Salvius, Holmiae, Stockholm.
- Mader L (1936) Beitrag zur Kenntnis der Hymenopteren, I. Entomologische Zeitschrift (Frankfurt a.M.) 50(23): 261–263.
- Madl M (1997) Über Vespidae, Pompilidae, Scoliidae und Tiphiidae der Türkei. Linzer biologische Beiträge 29(2): 823–827.
- Mauss V (2001) First record of *Polistes bischoffi* Weyrauch, 1937 from North Rhine-Westphalia with notes on the expansion of the distribution range of the species. Decheniana (Bonn) 154: 109–116.
- Mauss V, Treiber R (2004) Bestimmungsschlüssel für die Faltenwespen (Hymenoptera: Masarinae, Polistinae, Vespinae) der Bundesrepublik Deutschland, DJN, Hamburg, 2–53.
- Neumeyer R (2012) Polistes hellenicus Arens, 2011 auch in Kroatien gefunden. Ampulex 5: 15–18.

- Neumeyer R, Gigon A, Dobler Gross C (2011) Eine neue Feldwespe am Greifensee: Farbmorphe, Hybrid oder *Polistes gallicus* (Linnaeus, 1767)? Entomo Helvetica 4: 7–22.
- Nugroho H, Kojima J, Carpenter JM (2012 ["2011"]) Checklist of vespid species occurring in Indonesian Archipelago. Treubia 38: 71–186.
- Peters RS, Baur H (2011) A revision of the *Dibrachys cavus* species complex (Hymenoptera: Chalcidoidea: Pteromalidae). Zootaxa 2937: 1–30.
- Pimentel RA (1979) Morphometrics. The multivariate analysis of morphological data. Kendall-Hunt, Dubuque, Iowa, 276 pp.
- Praz CJ, Müller A, Danforth BN, Griswold TL, Widmer A, Dorn S (2008) Phylogeny and biogeography of bees of the tribe Osmiini (Hymenoptera: Megachilidae). Molecular Phylogenetics and Evolution 49: 185–197. doi: 10.1016/j.ympev.2008.07.005
- R Development Core Team (2013) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, http:// www.R-project.org
- Rambaut A (2009) FigTree v1.3.1: Tree Figure Drawing Tool. http://tree.bio.ed.ac.uk/software/figtree/
- Reeve HK (1991) Polistes. In: Ross KG, Matthews RW (Eds) The Social Biology of Wasps. Cornell University Press, Ithaca, 99–148.
- Rencher AC (2002) Methods of multivariate statistical analysis (second edition). Wiley, New York, 708 pp. doi: 10.1002/0471271357
- Richards OW (1973) The subgenera of *Polistes* Latreille (Hymenoptera, Vespidae). Revista brasileira de Entomologia 17: 85–103.
- Schlick-Steiner BC, Steiner FM, Seifert B, Sanetra M, Dyreson E, Stauffer C, Christian E (2010) Integrative taxonomy: A multisource approach to exploring biodiversity. Annual Review of Entomology 55: 421–438. doi: 10.1146/annurev-ento-112408-085432
- Schmid-Egger C, Treiber R (1989) Die Verbreitung von *Polistes bischoffi* Weyrauch, 1937 in Süddeutschland. Linzer biologische Beiträge 21: 601–609.
- Schneider N, Olinger F, Cungs J (1998) Éléments pour un catalogue des Hyménoptères Aculéates du Luxembourg. Bulletin de la Société des Naturalistes Luxembourgeois 99: 159–164.
- Seifert B (2008) Removal of allometric variance improves species separation in multi-character discriminant functions when species are strongly allometric and exposes diagnostic characters. Myrmecological News 11: 91–105.
- Stamatakis A, Ludwig T, Meier H (2005) RAxML-III: a fast program for maximum likelihoodbased inference of large phylogenetic trees. Bioinformatics 21: 456–463. doi: 10.1093/ bioinformatics/bti191
- Swofford DL (2002) Paup. 4.0b10 for Macintosh. Sinauer Associates, Inc., Sunderland, Massachusetts.
- Tolman T, Lewington R (1997) Butterflies of Britain and Europe. HarperCollins, New York, 322 pp.
- Villemant C, Simbolotti G, Kenis M (2007) Discrimination of *Eubazus* (Hymenoptera, Braconidae) sibling species using geometric morphometrics analysis of wing venation. Systematic Entomology 32: 625–634. doi: 10.1111/j.1365-3113.2007.00389.x

- West-Eberhard MJ (1969) The social biology of polistine wasps. Miscellaneous Publications of the Museum of Zoology, University of Michigan 140: 1–101.
- Weyrauch W (1937) Zur Systematik und Biologie der Kuckuckswespen Pseudovespa und Pseudopolistes. Zoologische Jahrbücher (Jena), Abteilung für Systematik, Ökologie und Geographie der Tiere 70: 243–290.
- Weyrauch W (1938) Zur Systematik und Biologie der palaearktischen Polistinen. Arbeiten über physiologische und angewandte Entomologie aus Berlin-Dahlem 5(3): 273–278.
- Weyrauch W (1939) Zur Systematik der paläarktischen Polistinen auf biologischer Grundlage. Archiv für Naturgeschichte, Neue Folge 8(2): 145–197.
- Wickham H (2009) ggplot2: Elegant graphics for data analysis (Use R!). Springer, New York, 212 pp.
- Witt R (2009) Wespen. Vademecum-Verlag, Oldenburg, Germany, 400 pp.
- Zhang DX, Hewitt GM (1996) Assessment of the universality and utility of a set of conserved mitochondrial COI primers in insects. Insect Molecular Biology 6: 143–150. doi: 10.1111/j.1365-2583.1997.tb00082.x
- Zimmermann K (1931) Studien über individuelle und geographische Variabilität paläarktischer *Polistes* und verwandter Vespiden. Zeitschrift für Morphologie und Ökologie der Tiere 22(1): 173–231. doi: 10.1007/BF00417968
- Zirngiebl L (1955) *Polistes* der Bayrischen Staatssammlung. Mitteilungen der Münchner Entomologischen Gesellschaft 44/45: 379–392.