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Clarification of *Einfeldia* Kieffer, 1922 (Diptera: Chironomidae) with *E. australiensis* (Freeman, 1961), comb. n. based on immature stages

PETER S. CRANSTON^{1,5}, JON MARTIN², MONICA MULDER³ & MARTIN SPIES⁴

¹*Evolution, Ecology & Genetics, Research School of Biology, Australian National University, Canberra, A.C.T. 2601, Australia.*
E-mail: pcranston@gail.com

²*Genetics, Genomics & Development, School of Biosciences, University of Melbourne, Melbourne, Australia 3010.*
E-mail: j.martin@unimelb.edu.au

³*Projects and Services, Sydney Water, 51 Hermitage Road, West Ryde, NSW 2114, Australia.*
Monica E-mail: Mulder@sydneywater.com.au

⁴*SNSB—Zoologische Staatssammlung München, Münchhausenstr. 21, 81247 München, Germany.*
E-mail: spies@zi.biologie.uni-muenchen.de

⁵*Corresponding author*

Abstract

The immature stages are described for the first time for *Chironomus* (*Xenochironomus*) *australiensis* Freeman (Diptera: Chironomidae) and the adult male is redescribed including from type specimens. The species does not belong to *Chironomus* Meigen or *Xenochironomus* Kieffer, but is best placed in a modestly expanded *Einfeldia* Kieffer. Application of this genus name is clarified, including by a lectotype fixation for its type species, *E. pectoralis* Kieffer, 1924. *Einfeldia australiensis* (Freeman) **comb. n.** provides the first record of the genus from Australia; otherwise the genus is reported confidently only from North America, Central America and western Europe to Japan. The immature stages of *E. australiensis* occur in relatively shallow mesotrophic to eutrophic dune lakes and maars with circum-neutral pH and high conductivity, from southeastern Queensland to southern Australia. The cytology is described briefly from larval salivary glands. Alternative genus placements for the species are discussed, and problems with *Einfeldia* and connected systematics in the tribe Chironomini are addressed.

Key words: Lectotype, Stability, new species, Australia, Biomonitoring

Introduction

In cataloguing the Austro-Pacific Chironomidae, Cranston & Martin (1989) had to leave several described species unallocated to a modern generic concept. Amongst these taxa was *Chironomus* (*Xenochironomus*) *australiensis* Freeman, 1961, described as adult males from four localities in south-east Australia. As contemporarily defined (e.g. Cranston *et al.* 1989), *Xenochironomus* Kieffer, 1921 lies outside *Chironomus* Meigen, 1803 and is accorded generic rank. Cranston & Martin (1989) placed *C. australiensis* in ‘incertae sedis’ because only the adult had been described, although the species had been reared from South Australia in 1973 (by Martin). Subsequently both Martin and Cranston recognised that its immature stages belonged to neither *Chironomus* nor *Xenochironomus*. A relationship to *Einfeldia* as exemplified by species group A of Pinder and Reiss (1983, larva; 1986, pupa), specifically by the European species *E. pagana* (Meigen, 1838), was suspected. This placement was accepted also by F. Reiss (in a letter to Cranston, June 1986) after his examination of reared Australian specimens. Understanding of the generic concept was impeded when ‘*Einfeldia*’ was rendered paraphyletic by inclusion of one or more species now belonging to subgenera of *Chironomus*. Problems with questionable allocation of species to the genus undoubtedly remain (e.g. Yamamoto *et al.* 2015), including the likelihood that most if not all identifications as ‘*Einfeldia*’ in GenBank to do not belong to the genus as understood here (Martin pers. obs.). However, *C. australiensis* does belong to *Einfeldia* as restricted recently (e.g. Epler *et al.* 2013), and here we propose it as the sole Australian chironomid allocated to the genus.

Why the identity of *E. australiensis* remained vague in subsequent studies of Australian Chironomidae (e.g. Cranston 1996; Bugledich *et al.* 1999; Madden 2010) is unclear. One complication was that *Chironomus vitellinus* Freeman, 1961 was allocated in error to *Einfeldia* by Cranston & Martin (1989). The name is a junior synonym of *Chironomus javanus* Kieffer, 1924; thus, the species remains in *Chironomus*, although it is somewhat atypical there. Here we rectify regional confusion with a redescription of the adult male of *C. australiensis*, provide first descriptions of the immature stages, and briefly describe the cytology. We designate a lectotype for the European *E. pectoralis* to stabilise the genus name, and then we discuss the wider implications for delimitation of *Einfeldia*. Ecological notes assist in understanding the potential significance of *E. australiensis* in biological monitoring of Australian aquatic ecosystems.

Methods

Rearings of *E. australiensis* were undertaken (by Martin) from individual larvae. Unreared pupal exuviae were skimmed from lake surfaces, and larvae collected by kick sampling. Slides were made according to standard protocols into Euparal and are preserved in the Australian National Insect Collection (ANIC), CSIRO, Canberra, Australia, unless stated otherwise (SW, Sydney Water). Material and documents at the Zoologische Staatssammlung München (ZSM) in Munich, Germany were examined by M. Spies. Morphological terminology follows standard references (larva, Epler *et al.* 2013; pupa, Pinder & Reiss 1986; adult male, Cranston *et al.* 1989). Measurements are in µm unless stated otherwise (mm). Cytological preparations were made in lactic acetic orcein by the usual method of Martin *et al.* (2006).

Results

Einfeldia Kieffer

Einfeldia Kieffer, 1924:393-Kieffer (1924), adult male and female.

“*Einfeldia* species group A”—Pinder & Reiss (1983), larva; (1986), pupa.

Einfeldia Kieffer—Cranston *et al.* (1989), adult male; Epler *et al.* (2013) *partim*, larva.

'so-called *Einfeldia pagana*-group—Yamamoto *et al.* (2015), adult male, larva.

Typification. Application of the name *Einfeldia* Kieffer has undergone several significant changes. The reasons for this include that none of Kieffer's adult syntypes for the type species, *E. pectoralis* Kieffer, 1924, has ever been seen or found again by anyone else, and that no other name-bearing type specimen was recognised or designated. Moreover, the type series possibly was mixed; see Discussion below. Therefore, the following lectotype fixation is made to promote nomenclatural stability and facilitate taxonomic diagnostics.

Lectotype here designated for '*Einfeldia pectoralis*' (original label on the slide) Kieffer, 1924; in coll. SNSB – Zoologische Staatssammlung München (Germany): ♂ pupal exuviae, slide-mounted in Euparal (F. Reiss); GERMANY, Schleswig-Holstein, Großer Waterneverstorfer Binnensee, reared from sample 'W.S.Z. bei 6' (original label on slide) taken at 1.5–2.5 m depth, 19.vi.1923, *leg.* F. Lenz.

There is 'firm evidence' that the pupal exuviae designated above is 'biologically and for the purposes of nomenclature' (ICZN 1980) part of one of the syntypes of *E. pectoralis* that Kieffer (1924) had described as adults from Lenz's rearings (see Lenz 1937). In the voluminous correspondence on such cases between A. Thienemann / F. Lenz and J.J. Kieffer that is preserved at ZSM (see, e.g., Spies 2001) there is one letter, dated '27.ix.1923' by Thienemann, that includes an identification request for a sample 'W.S.Z. bei 6' (in Lenz's handwriting); the latter sample code appears nowhere else in the correspondence. When Kieffer returned that letter, he had entered his identification results for that particular sample as '*Fleuria lacustris* ♂♀ Viele Exemplare' [many specimens] and '*Einfeldia pectoralis* n.sp. 1 ♂'. Chironomid material at ZSM labelled with Lenz's sample code 'W.S.Z. bei 6' includes only specimens under these two names from Kieffer, and no specimen under the name *Einfeldia pectoralis* other than the single ♂ exuviae here designated as lectotype. The adult males and pupae of the two species are so different that life-stage misassociation can be ruled out in this case. Therefore, the single exuviae labelled '*Einfeldia pectoralis*' is recognisable beyond reasonable doubt as part of one of Kieffer's syntypes in accordance with ICZN (1980) Opinion 1147. Consequently, this exuviae is eligible for lectotype fixation.

On the letter sheet mentioned above, Kieffer recorded *Einfeldia pectoralis* in two more of Lenz's original sample rearings ('W.S.Z. 10–11a', 'W.S.Z. 10–11b'), also from Waterneverstorfer Binnensee. Three ♂ exuviae at ZSM (1 slide-mounted by Lenz, the others on another slide by Reiss) apparently belong to these samples. However, Kieffer's letter entries recorded the respective specimens as '♂♀' and '1♂ 1♀', and the sample code on the slide labels is 'W.S.Z. 10–11' without a subsample letter a or b. Thus, these 3 exuviae cannot be ascribed type status, as the individual life-stage association required (ICZN 1980) is impossible. Similarly, the few specimens at ZSM of larvae assigned to *E. pectoralis* by Lenz (1937, 1954 in 1954–1962) are not associable with the certainty that would be necessary to interpret them as parts of original type specimens.

Species (names) included

Einfeldia atitlanensis Sublette & Sasa, 1994—male and female described from Guatemala

Einfeldia australiensis (Freeman, 1961)—revised below

Einfeldia pagana (Meigen, 1838)—requires Holarctic revision

Einfeldia pallida Lenz, 1937—*nomen dubium*

Einfeldia pectoralis Kieffer, 1924—requires revision

Einfeldia sp. A—Epler (2001; larva in key), Epler *et al.* (2013; larva in genus diagnosis). Judging from specimens seen by M. Spies (two individual associations of larval skin + pupal exuviae + adult male; USA, Georgia, Brooks Co., wetlands along Hwy 33, 13.v.1992/03.ix.1992, *leg.* B. A. Caldwell), this is a separate species that remains to be described and named.

For species removed from *Einfeldia* s. str. recently, see Discussion: Delimitation of *Einfeldia* Kieffer.

Einfeldia australiensis (Freeman)

Chironomus (*Xenochironomus*) *australiensis* Freeman, 1961: 696 (adult male).

Type material. **Holotype** ♂, pinned, genitalia on celluloid, foreleg, head and antennae on separate slide in Euparal; AUSTRALIA: New South Wales, Oxford Falls, 3.iv.1922 (collector not stated) (ANIC). **Paratypes** (slide mounted in Euparal, Cranston): ♂, as holotype; ♂, N.S.W., Hornsby, 9.i.1958 (*Lee*).

Other material [all AUSTRALIA, and in ANIC unless stated otherwise]: Pe, Queensland, Fraser Island, Ocean Lake, 24°55.5'S 153°16.4'E, 2.vi.1997 (*Wright, Reid, Gunn*); 4L (3 slides), New South Wales, Sydney, Botany Wetlands, Pond #3, 33°56'08"S 151°12'53"E, 2.xi.2015, Sydney Water (SW) via Monica Mulder (2 slides, 1 larva on each; 1 slide, 2 larvae, with *Procladius* under 2 other coverslips (SW); 2L (one with chromosome squash) Blue Lagoon, 29°58'S 153°15'E, 15.viii.1976 (*Timms*); 2Pe, Australian Capital Territory, Jervis Bay, Lake McKenzie, 35°09'S 150°41'E, 28.iv.1997 (*Wright*); Pe, same except 7.iii.1998; Pe, Victoria, Lake Little Beatle, 37°47.2'S 148°25'E, 17.xii.1996 (*Wright*); Swan Lake, 38°12'S 141°19'E, 20.xii.1996 (*Wright*); South Australia, Valley Lake, Mt. Gambier, 37°30'S 140°45'E, 15.viii.1972 (*Timms*), 'ASA.34.2'; L (with chromosome squash) (Museum of Victoria); 3L (with chromosome squash), Le/Pe/♂, Pe/♂, P(♂), Lake Edward via Kalangadoo, 37°38'S 140°35'E, 30.ix.1973 (*Martin & Martin*), 'ASA 36.3'.

Excluded from *E. australiensis* here (all = *Xenochironomus* spp.; see 'Remarks on Freeman's type series' below). Paratype, ♂, pinned, genitalia on celluloid, AUSTRALIA: [A.C.T.] Molongolo R., .iii.1930 (*Graham*). 2♂♂, 1♀ slide mounted in Euparal, several ♂, ♀, pinned, Northern Territory, Jim Jim Ck., WSW of Mt. Cahill, 'at light', 24.x.1972 (*Colless*).

Remaining four ♂ paratypes listed by Freeman (1961) not examined.

Description. Adult male (Figs 1A, C, D; 2), (n=4, slide mounted, variously incomplete) [statements in square parentheses from Freeman (1961)].

Colour (dry, pinned) and size. Thorax yellow-brown with scarcely darker vittae; legs green, foreleg with darker tibia and tarsomeres, mid and hind legs with dark tarsomeres; abdomen light green becoming darker posteriorly [pale green, yellowish-red thoracic vittae, knees somewhat darkened, legs pale green, abdomen clear green]. Total length 5.0–6.5 mm, wing length 2.2–2.9 mm (from arculus to tip) [2.75–3.5 (from origin at thorax to tip)].

Head. Antenna with 11 flagellomeres, fm 1–10 length 155–175 µm, fm 11 475–520; AR 2.7 ['about 3'].

Frontal tubercle globular, 5–7 across [absent], without apical seta. Head with 15–26 irregularly biserial and continuous verticals + postorbitals; clypeus square, with 15–18 clypeals; palpomere lengths (2nd–5th) 50–65; 120–175; 150–200; 210–320; 4–6 linearly arranged sensilla chaetica on 3rd segment, not arising from pit.

Thorax. Anteprenotal lobes quite narrow dorsally and fused at shallow notch (Fig. 1A), without anteprenotals; mesonotum with slight indication of scutal tubercle; acrostichals 19–21 in irregularly biserial row starting near anteprenotum, 6–11 uniserial dorsocentrals, 5–6 long prealars, c. 16 biserial scutellars of 2 sizes.

Legs variously damaged: Leg Ratios: foreleg 1.57–1.65 [nearly 2], mid leg 0.46–0.48, hind leg 0.55–0.6. Foreleg BV ratio 0.63, SV ratio 0.65 (both from holotype only). Fore tarsal beard sparse, BR 5.0 on tarsomeres 1 and 2. Fore tibial scale low, rounded without spur (Fig. 1C); each mid and hind tibial apex with wide and narrower comb, each comb with 25 long spur (Fig. 1D). Tarsal sensilla chaetica ($n=1$) 26 linear in distal 2/3 of ta_1 of mid leg, 13 in distal 1/4 of ta_1 of hind leg. Fore tarsomere 5 cylindrical. Pulvilli strong, subequal to claw.

Wing generally as in Cranston *et al.* (1989: fig. 10.19B), plain, membrane with only fine punctation; R_{2+3} running close to R_1 , ending close to apex of R_1 , substantially prior to wing apex; R_{4+5} and C ending together near wing apex, without costal extension. Wing vein setation: R with 25–33, R_1 with 17–26 along complete length, R_{4+5} with 20–34 in distal half; 8–16 squamals. VR (ratio cubitus to media) 1.07.

Genitalia (Fig. 2). Laterosternite IX without setae. Tergite IX with anal tergite bands moderately developed, widely separated and ending distally near origin of anal point. Fig. 2A, C); dorsomedial TIX setae in pair of clusters between the tergite bands, 11–20 setae in each cluster. Anal point (Fig. 2A, C) arising anteriorly of posteroventral end of tergite IX, very broad and variably curved ventrad (Fig. 2A) such that its length can be impossible to measure in dorsal view; apparently T-shaped in cross-section; dorsal surface bare. Superior volsella (Fig. 2A–C) in dorsal view trapezoidal or pad-like, entirely trichiose (not shown in Fig. 2B, bottom), with variably located shorter to longer setae on dorsal, medial, caudal and lateral faces; volsella laterally with variably developed short, subacute process. Inferior volsella (Fig. 2C, D) elongate, parallel-sided, not clubbed distally, microtrichiose and with many distal setae, but without any special terminal seta. Gonocoxite 185–210 long, with medial row of 4–5 setae (Fig. 2D). Gonostylus 175–220 long, slightly widened in mid-section, its tip tapering slightly, medially bearing 8–10 stronger, clustered setae (Fig. 2C). Major internal apodemes of segment IX as in Figure 2D.

Female unknown.

Pupa (Fig. 3) ($n=8$). Length c. 7 mm, medium brown, with weak abdominal apophyses.

Cephalothorax. Frontal warts absent. Cephalic tubercle (Fig. 3A) broadly conical, c. 75 long, with short (20 μ m), pale frontal seta inserted slightly subterminally. Thorax rugose especially anterodorsally, with weak scutal tubercle. Thoracic horn multi-branched, hyaline, arising from double, medially narrowly divided tracheal plate in medially slightly constricted basal ring (Fig. 3B). Prealar area with two projecting lobes, anterior lobe directed laterally, posterior one more posteriorly (Fig. 3C).

Abdomen. Tergal armament (Fig. 3D): Tergite I with posterior transverse fine shagreen; TII–VI more broadly and densely spinulose in near continuous central patch, including pair of posteromedial patches with larger points (Fig. 3D); spinulose area larger on more posterior segments; TVII with anterolateral areas of fine spinules, TVIII–IX bare. Hook row on TII undivided, with 55–60 uniserial hooks, extending c. 50% width of segment. Paratergites II–IV spinulose, others bare. Conjunctives all bare. Sternite I with no lateral or medial anterolateral tubercles, with sparse fine posterior spinulation. SII with posteromedial pattern of short to elongate and strong but translucent spines (Fig. 3E). Remaining sternites bare. Posterolateral 'comb' of segment VIII (Fig. 3G) of 5–7 brown, basally separated, tapering spines c. 80–110 long. Anal lobe with dense, uniserial fringe of 70–80 taeniae, with single dorsal seta inserted quite anterolaterally. Pedes spurii A on SIV strongly developed. Pedes spurii B well-developed on II, absent on III.

Setation: 1 pair of anterolateral O setae each on tergites II–VIII and apparently on at least anterior sternites. Tergite I with 2 D setae, II with 4, III–VII with 5, VIII with 1. Sternite I with 2 V setae, II–VII with 3–4, VIII with 1. L setae on I–IV semi-taeniate, 1 on I, 3 on II with the L3 located ventrally on bases of pedes spurii B; 3 each on III and IV, the L3 exceptionally on respective conjunctive. 4 taeniate L setae each on V–VIII.

Larva (Fig. 4). Length c. 7–12.5 mm, head capsule length c. 660–700, postmentum length 270–280. Head pale yellow, with occipital margin, teeth of mentum, ventral teeth of mandible and premandibular teeth variably contrasting brown. Eyespots double, dorsal spot above or slightly anterior of ventral one, both more or less circular. Dorsal surface of head (Fig. 4A) including a fused frontoclypeus with cephalic setae S5 inserted anteromedially of dorso-lateral pits, with large, ovoid to heart-shaped fenestra between the S4, with S3 inserted subapically in broad

‘scalloped’ area representing fused clypeus, with convex anterior margin. Lateral sclerites granulate, fragmented and not individually distinguishable; S2 arising in that area near posterolateral corners of labral sclerite. Labral sclerite strongly widened anteriorly, with S1 arising near the mid-lateral bend.

Antenna (Fig. 4B) 5-segmented with segment lengths 88–93: 25–28: 12–15: 12–15: 7; AR 1.4–1.6; Lauterborn organs slender, opposite, 10 long; style slender, 10 long; blade 65–70 long, extending to antennal apex. Labro-epipharyngeal region (Fig. 4C, D): SI setae finely plumose along most of both edges, SII simple. Pecten epipharyngis comprising three indistinctly separated scales, each scale with 4–8 teeth of somewhat variable shape, size and level of origination on plate (Fig. 4D), premandible 100–105 long, with 2 long apical teeth and two short, partially fused proximal teeth; brush extensive. Mandible (Fig. 4E, F) 215–225 long, with short, pale brown dorsal tooth and small (15 long), more proximal accessory dorsal tooth (Fig. 4F); apical tooth and three inner teeth dark, innermost tooth clearly delimited from mola; seta subdentalis 15 long, hooked apically; mola smooth, without spinules on medial edge. Maxilla (Fig. 4G) with chaetulae of palpiger c. 15 long.

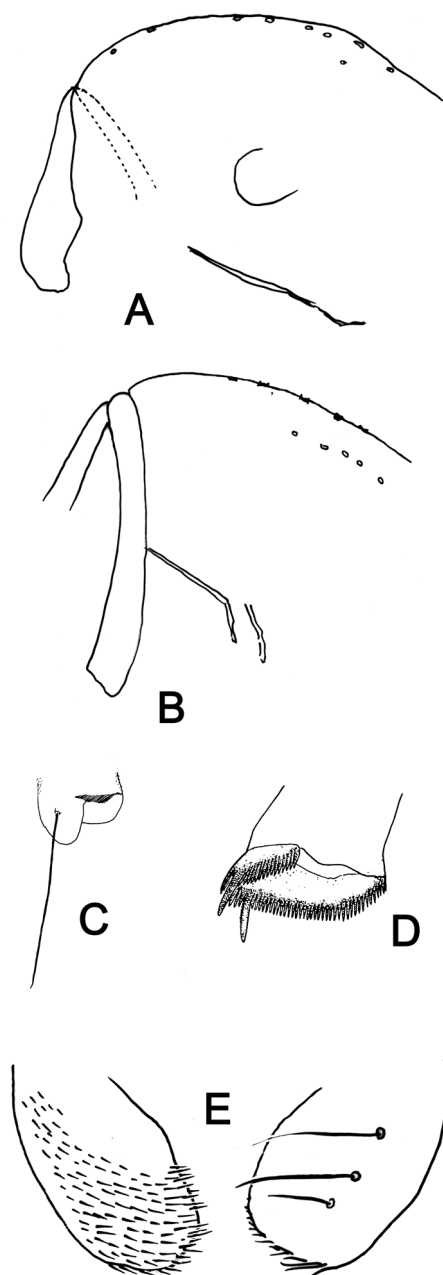


FIGURE 1. Adult male. A. *Einfeldia australiensis*, anterior thorax, lateral view; B. *Xenochironomus* sp., anterior thorax, lateral; C, D. *Einfeldia australiensis*, fore and hind tibial apices; E. *Xenochironomus* sp., superior volsella: left = dorsal; right = ventral.

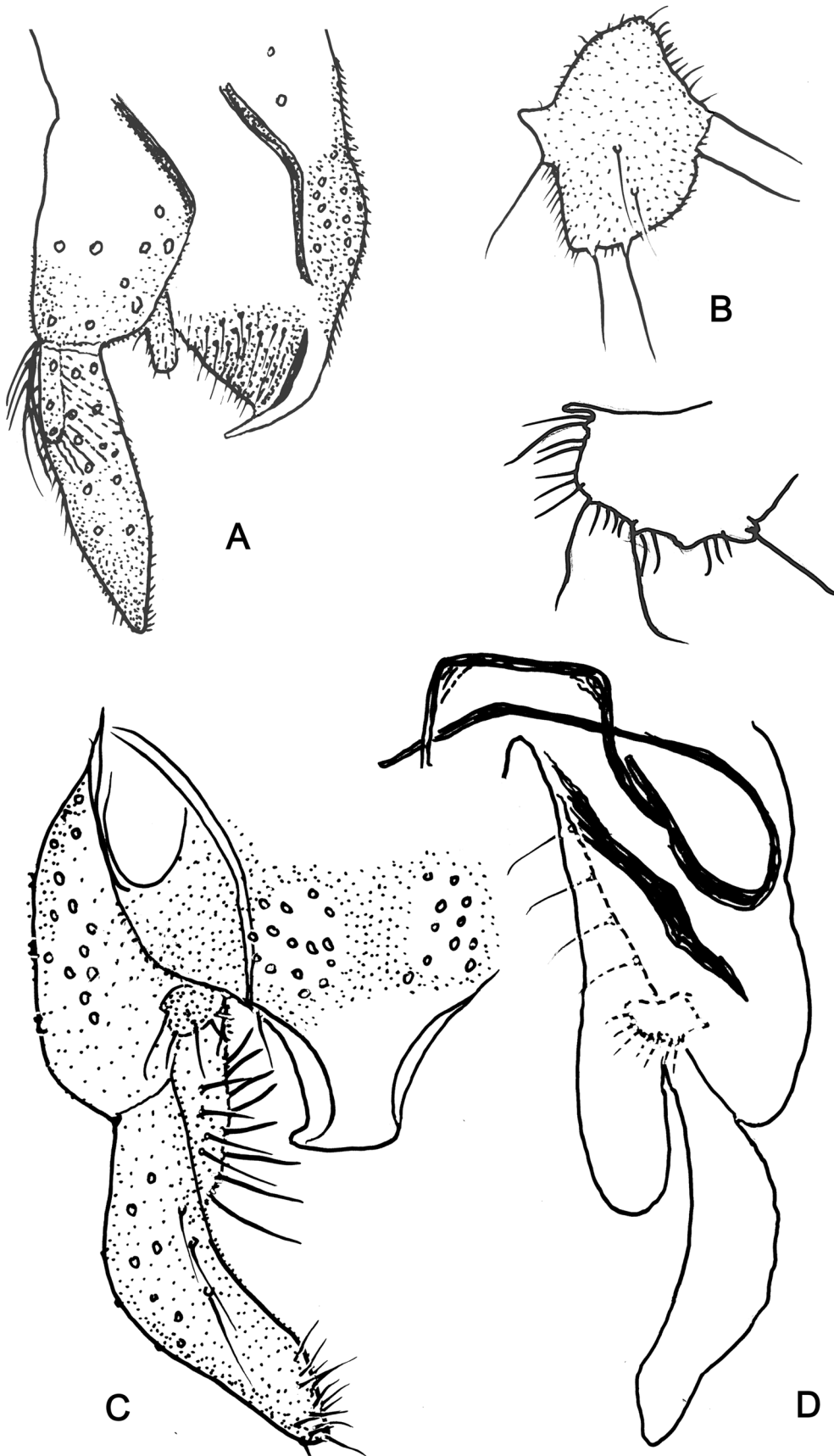


FIGURE 2. Adult male genitalia, *Einfeldia australiensis*. A. Hypopygium, lateral. B. Superior volsella (variants). C. Hypopygium, dorsal. D. Hypopygium, stylized ventral/internal.

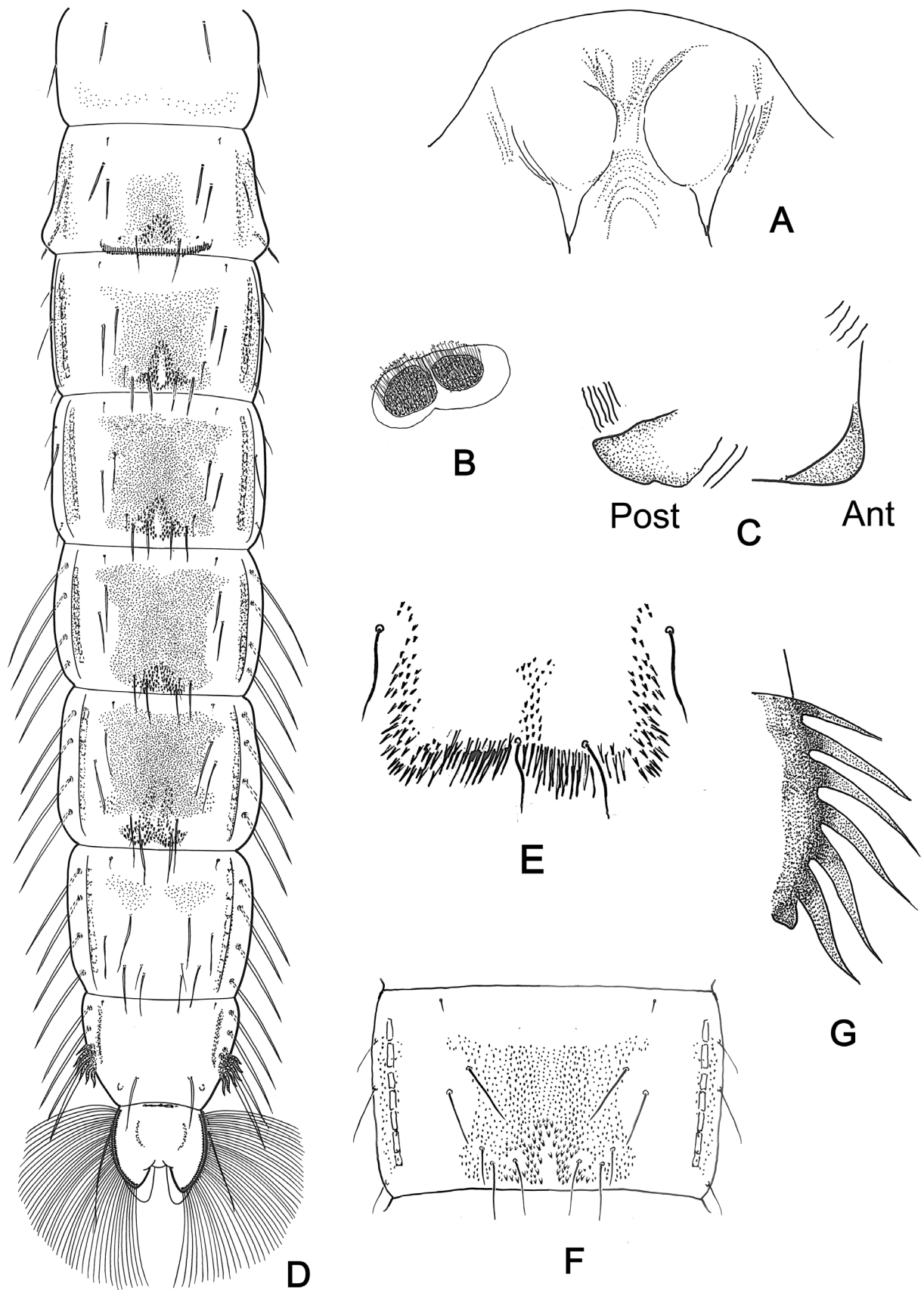


FIGURE 3. Pupa, *Einfeldia australiensis*. A. Cephalic tubercles; B. Basal ring and tracheal plate; C. Prealar tubercles: Ant = anterior; Post = posterior. D. Abdomen, dorsal. E. Sternite II, armed postero-median section. F. Segment III, dorsal. G. Posterolateral part of parasternite VIII, 'comb'.

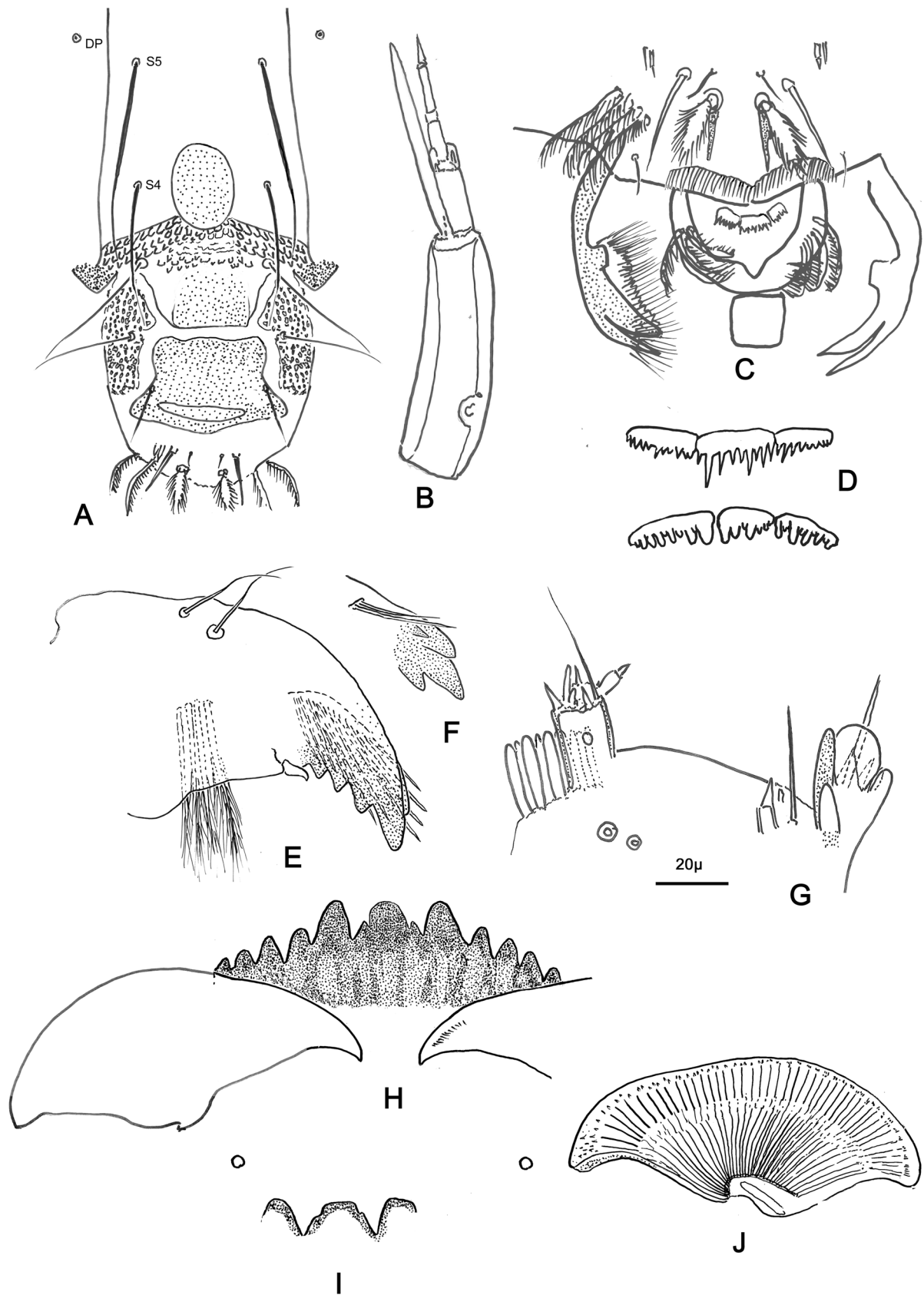


FIGURE 4. Larva, *Einfeldia australiensis*. A. Head capsule, frontal (top) to labral region: DP = dorsal pit; S4, S5 = cephalic setae; B. Antenna; C. Labro-epipharyngeal region; D. Pecten epipharyngis, variants; E. Mandible, ventral; F. Mandible apex, dorsal; G. Maxilla; H. Mentum; I. Medial teeth on worn mentum; J. Ventromental plate.

Mentum (Fig. 4H, I) 155–160 wide; single median tooth domed, with pair of variably visible lateral accessory teeth (strongly worn in Fig. 4I); each side of mentum with six lateral teeth evenly decreasing in size. Ventromental plate (Fig. 4J) 165–175 wide, 80 deep, with 40–46 striae extending near full depth and across complete width of plate, each stria terminating anteriorly in 1–2 spinules slightly posterior to plate margin.

Abdomen. Anterior parapod with dense cluster of pale golden, simple claws. Abdominal segment VIII with one pair of curved or partially coiled ventral tubules c. 1.1–1.6 mm long. Procerci and posterodorsal ('supraanal') setae of segment IX pale to medium brown, procercus with 6–7 brown, c. 800 long anal setae. Posterior parapods 390–420 long, claws golden. Anal tubules pale, sausage-shaped without constriction, c. 2x as long (250–320) as wide.

Cytology. The karyotype comprises three polytene chromosomes (Fig. 5). Comparison to the karyotypes of two North American *Einfeldia* species (Martin 2016) indicates that this is derived from a typical $2n=4$ karyotype, comprising seven chromosome arms, common to most species of *Chironomus* (s. l.) (Michailova 1989, Martin 1996), *i.e.* the small acrocentric chromosome is fused to the end of one arm of a metacentric chromosome. The banding pattern cannot be compared directly with those of *Chironomus* (Keyl 1962, Devai *et al.* 1989), although there are some hints of general homology for some chromosome arms. Almost certainly the terminal segment bearing two Balbiani rings (BRs) is the equivalent of arm G and this may be fused to the equivalent of arm E. The position of the centromere is unclear in this chromosome, whereas in the other two chromosomes there are heterochromatic bands that are likely to represent the centromeres. The nucleolus is near the middle of the arm of one of these two chromosomes. No polymorphisms were present in the studied individuals.

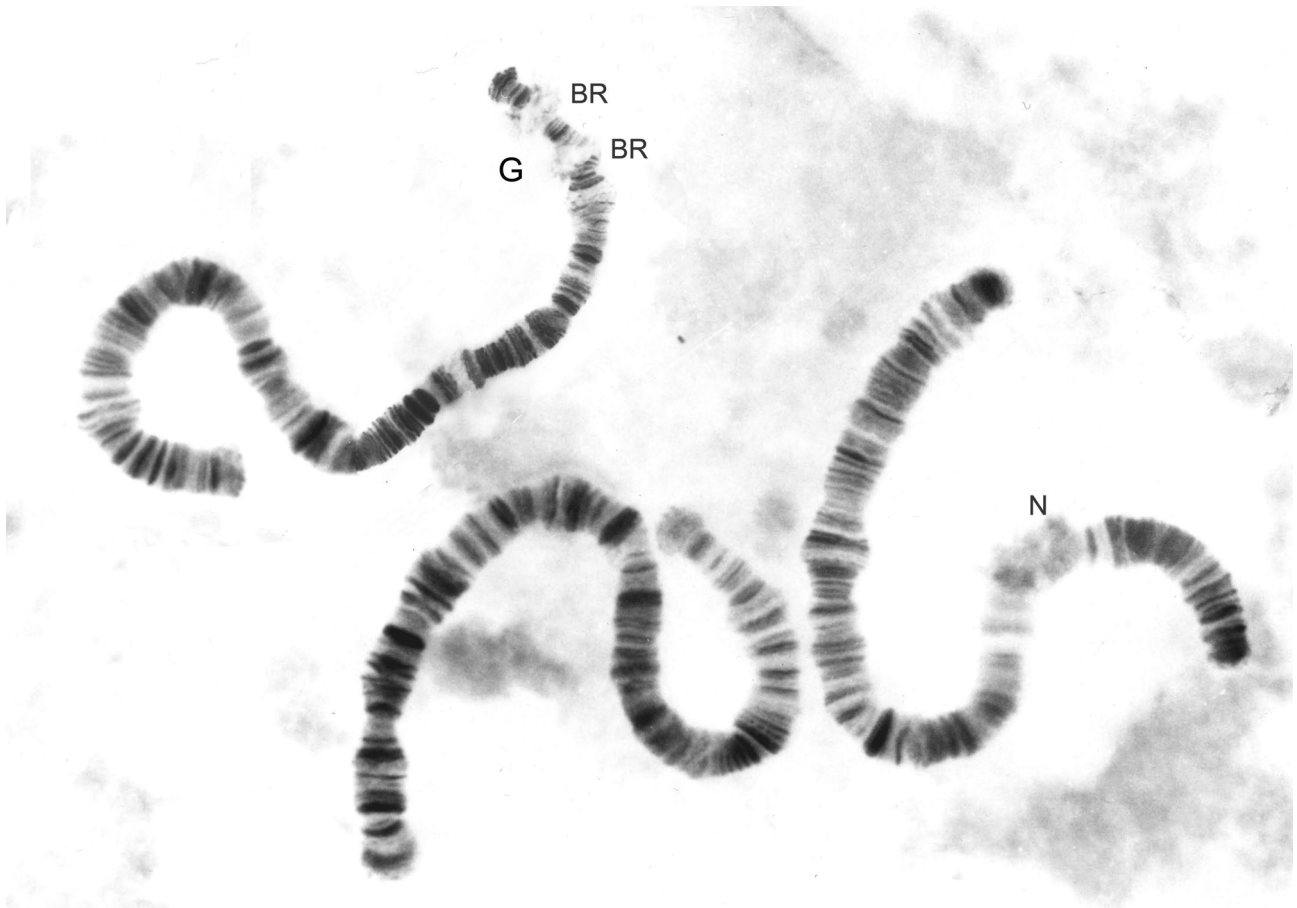


FIGURE 5. Cytology, *Einfeldia australiensis*. Polytene chromosome complement: BR = Balbiani ring; G = presumed homologue of arm G in *Chironomus* species; N = nucleolus.

Ecology and significance to biomonitoring. Enough records of the immature stages of *E. australiensis* have been found to allow a summary of the distribution and assess the types of water bodies in which they live. Pupal records dominate—the stage is distinctive, easily collected and has been recognised in lentic studies (*e.g.* Wright & Cranston 2000, Wright & Burgin 2007). In South Australia, Lake Edward is a productive maar lake with surface

area 29 ha, maximum depth 7 m and mean depth 4.2 m, probably unstratified, with black mud sediment comprising 46.6% organic matter, 30% carbon and 1.8% nitrogen (Timms 1974). In this lake, larvae of '*X. ? australiensis*' were found in one of eight samplings amongst weed at a depth of 1.5 m (*leg. et det.* J. Martin, August 1973). The same collector reported larvae also from nearby Valley Lake (37°51'S; 140°46'E) at a depth of 10 m and a density of 17 individuals m⁻¹.

In subcoastal northern New South Wales, larvae of '*X. ? australiensis*' were reported by Timms (1982) from Blue Lake, a freshwater lake with marine contact and variable water chemistry according to season and status of the marine connection. Further south in Jervis Bay, Lake McKenzie is a permanent, closed, variably shallow (max. 9 m deep) freshwater dune lake of 9 ha, with pH 5.5–6.5, relatively dilute (160–230 µS/cm), with P 30 µg/l and N below detection limits, and with thermal stratification when sampled. Although '*X. australiensis*' was not reported from there by Norris *et al.* (1993), exuviae were found by Wright & Cranston (2000) to be common in late austral summer (April) but absent in cooler September.

Recently larvae were collected from Botany Wetlands, eastern Sydney, where sediment conditions are essentially sand with a thin layer of biological matter. The water was slightly turbid at the time of collection but usually it is quite clear for a 'wetland'. Conductivities in the connected Pond 5 and Mill Pond typically range between 100 and 400 µS⁻¹ but reached 530 µS.cm⁻¹ on occasion, indicating an intermittent marine connection. High conductivities, suggesting marine incursions, are noted also at Ocean Lake, a disturbed dune lake on Fraser Island, Queensland, where conductivity was 365 µS.cm⁻¹ and pH 6.3 (Ian Wright, pers. comm. 2016).

In summary, the immature stages of *E. australiensis* occur in relatively shallow mesotrophic to eutrophic, circum-neutral pH and high-conductivity dune lakes and maars, from southeastern Queensland to southern Australia.

Remarks on Freeman's type series. The type series of *C. (X.) australiensis* comprises pinned adults, several of them with a genitalia preparation on a celluloid mount associated with the pinned specimen (*i.e.* on the same pin). The holotype is located in ANIC, as are several paratypes, including from the type locality, Oxford Falls, NSW. Freeman (1961) stated the holotype and 3 paratypes to be in 'SPHTM' (the School of Public Health and Tropical Medicine, University of Sydney), but this collection has been de-accessioned and divided between other institutions, including ANIC. Some other paratypes were listed as in CSIRO, but not all of these are present there.

The extant material is mixed: the holotype and some paratypes belong to *Einfeldia*, but at least one paratype (from Molongolo R.) belongs to an undescribed species of *Xenochironomus*. A series of adults from Northern Territory, identified subsequently by D.H. Colless, all belong to *Xenochironomus*. The respective adults resemble each other, differing subtly as elaborated below, but can be separated as follows. The anteprenotal lobes are narrowly fused medially in the *Einfeldia* specimens, whereas they make slight contact but are weakly separated in the *Xenochironomus*; frontal tubercles are present in the *Einfeldia*, absent in the *Xenochironomus*; the superior volsella is subrectangular with a small posterolateral projection in the *Einfeldia*, but tongue-shaped and without projection in *Xenochironomus*.

Transferring *C. (X.) australiensis* to *Einfeldia* does not remove the modestly diverse but rarely-reared genus *Xenochironomus* from Australia. Aside from the paratype and N.T. specimens discussed above, we are aware of adults of at least two unnamed species, several forms of pupal exuviae and larvae (Cranston 1996).

Discussion

Delimitation of *Einfeldia* Kieffer. The concept, contents and status of *Einfeldia* have been, and to an extent remain, confused. The genus was erected by Kieffer (1924) based on adult males and females he placed into a single species, *Einfeldia pectoralis* Kieffer, 1924, which is the type species by monotypy (Ashe 1983). Confusion commenced with the original publication in which Kieffer credited the genus name to Thienemann, who had suggested it in correspondence (preserved at ZSM). However, the agreement *in litteris* had been for *Einfeldia* to be established for a different species reared from Einfeld See (*Chironomus insolitus* Kieffer, 1922; see below). Kieffer (1924: 393) stated for *E. pectoralis*: 'Larve dans le même lac que l'espèce précédente', which was *Glyptotendipes scirporum* from 'lac d'Eutin'. In reality, however, Kieffer's adult types of *E. pectoralis* had not been reared from either of those two lakes, but from immature stages collected in Großer Waterneverstorfer Binnensee, a shallow, eutrophic and periodically slightly brackish lake near the German Baltic Sea coast northeast of Plön. This

fact was stated repeatedly by Lenz (1933, 1937, 1954 in 1954–1962) and is supported by corresponding entries in the letters exchanged between Thienemann/Lenz and Kieffer (see the Results section above).

In a letter reply to Lenz written just prior to the 1924 publication, Kieffer listed as belonging to *Einfeldia* the species *E. pectoralis*, *Chironomus insolitus* Kieffer, 1922, *C. insolitus* "var. scapularis" (a *nomen nudum*), *C. bequaerti* Goetghebuer, 1921 (currently a junior synonym of *C. (Lobochironomus) dorsalis* Meigen 1818), and *C. latidens* Goetghebuer, 1921 (currently a junior synonym of *Einfeldia pagana* (Meigen, 1838)). In a subsequent letter to Lenz written in 1925 Kieffer added another species found in Waterneverstorfer Binnensee, "*Einfeldia pallida* n. sp.". Kieffer died (in December 1925) without publishing on this, but the name *E. pallida* was made available when Lenz (1937) published it with the diagnosis of a pupa. Evidently, Kieffer's taxonomic circumscription of *Einfeldia* was wider than Thienemann's initial idea (*E. insolita*) and what Kieffer (1924) named *E. pectoralis*, but the latter is the only part that counts for nomenclature. Lenz's subsequent publications on *Einfeldia* (1929, 1933, 1937, 1954 in 1954–1962) essentially built on Kieffer's wide concept of the genus, in spite of Lenz noting differences among immature stages for which he gave the first diagnoses. Eventually, *Einfeldia sensu lato* of Lenz (1954 in 1954–1962) had come to include an 'insolita group' consisting of *E. insolita* and *E. dissidens* (Walker, 1856), a 'pectoralis group' comprising *E. pectoralis* and *E. pallida*, and a 'pagana group' consisting only of *E. pagana*. In today's chironomid system, Lenz's insolita group is reasonably equivalent to *Einfeldia* 'species group B' sensu Pinder & Reiss (1983, 1986) and to *Benthalia* Lipina, 1939 sensu Sæther & Spies (2013). The pectoralis and pagana groups combined approximately correspond to *Einfeldia* 'species group A' sensu Pinder & Reiss (1983, 1986) and to *Einfeldia* in the strict sense proposed by Epler *et al.* (2013). The delimitation of *Einfeldia* as defined by its type species becomes reproducible for the first time through the lectotype fixation herein for *E. pectoralis*. This now allows precise diagnoses for *Einfeldia* to be developed that should overcome long-standing instability and uncertainty due to the many and varied interpretations of this genus name in the literature since the 1920s.

Kieffer's (1924) adult syntypes of *E. pectoralis* must be considered as lost, but his description indicates that the series may have included members of more than a single species. For example, the frontal tubercles were described as 'cylindrical, more than 2 times as long as wide, with a long distal seta'. This condition resembles the cephalic tubercle with frontal seta on the pupae of many Chironomini, but is quite exceptional for corresponding imagines. Concerning members of genera in question here, we are aware of such tubercles only from adults of *Einfeldia pagana*, in which the configuration can range from a short tubercle with an apical macrotrichium (M. Spies, pers. obs.) through a tubercle carrying a slightly longer seta (Shilova 1980: 185 and fig. 8A₃; tubercle 'barely noticeable', length and width 6 µm) to a slightly elongate tubercle with a much longer seta (e.g. Oliver 1971: fig. 1, *sub E. synchrona*). Another element that points in the same direction is Kieffer's (1924) description of the tip of the gonostylus as having the distal-medial group of setae in '2 or 3 rows of 8 hairs that are rigid and not long'. In all members of *Einfeldia s. str.* these setae are arranged similarly, in a relatively irregular cluster (e.g. Shilova 1980 for *E. pagana*: 'Medial margin of gonostylus near apex densely covered with setae ([number] up to 14). These no longer than width of gonostylus at point of their attachment. '; for *E. australiensis*, see Fig. 2C). In contrast, on males with '*Einfeldia*-like' superior volsellae now placed in *Chironomus sensu lato* the distal-medial gonostylus setae are longer and uniserial (in a single row).

In contrast to the data in Kieffer (1924) that suggest *E. pectoralis* as close or even identical to *E. pagana*, his description of the adult male colouration, especially of the anal point and distal part of the superior volsella as 'black', is unmatched by what we have seen or read on *E. pagana*. In this aspect a species in *Chironomus (Lobochironomus)* would make a better fit. This may explain Hirvenoja's (1998) application of the name *E. pectoralis* to Finnish adults that we would place in *Lobochironomus*, not least because their brief diagnosis in Hirvenoja's key is incompatible with Kieffer's (1924) data for *E. pectoralis* concerning other characters. Examples here are the colouration of thoracic vittae, legs and abdomen, and the distal-medial gonostylus setae (few long ones in a single row in Hirvenoja 1998: fig. 7A).

The apparent discrepancies in Kieffer's (1924) description of adults in *E. pectoralis* imply two possible explanations. Either (A) the males in Kieffer's type series represented at least two taxa, or (B) all these males belonged to a single species close or identical to *E. pagana*, but their colour fell outside known variation, perhaps due to the influence of brackish water as reported for the type locality by Lenz (1933). Similar effects have been noticed (M. Spies, pers. obs.) but appear unstudied in detail. Whatever, these problems with Kieffer's (1924) description and type series of *E. pectoralis* underline that the lectotype fixation in the present work is warranted.

The species included in *Einfeldia* Kieffer in the present work are listed in the Results section on the genus. That list excludes the following species; for earlier removals, see Epler *et al.* (2013: 407).

Chironomus kanazawai (Yamamoto, 1996)—transferred by Yamamoto *et al.* (2015)

Chironomus ocellatus (Hashimoto, 1985)—transferred by Yamamoto *et al.* (2015)

Chironomus (Lobochironomus) palaearticus (Ashe, 1990)—transferred by Sæther & Spies (2013) upon examination of type material

?*Glyptotendipes (Heynotendipes) nojiriprimus* (Sasa, 1991)—excluded from *Einfeldia* here; present tentative placement strongly suggested by morphological data available to us. Moreover, Yamamoto *et al.* (2015) reported that the species was misidentified earlier as *Einfeldia chelonia* (Townes, 1945), which has been transferred to *Glyptotendipes (Heynotendipes)* upon examination of type material (see Epler *et al.* 2013: 407).

'Convergent' character states. Several morphological features previously considered as significant or informative to definitions of *Einfeldia sensu lato* (and to genus diagnostics in Chironomini) occur also in one or more species of other genus-group taxa, some of which are not related closely to *Einfeldia s. str.* Such convergences pose serious problems to phylogenetic reconstruction in general, and to generic placement of species like *E. australiensis*.

Concerning larvae, a large fenestra on the frontoclypeus in the region carrying the S4 setae occurs in all known larvae of *Einfeldia s. str.*, but also in some *Demeijerea*, in *Dicrotendipes lobiger* (Kieffer), and in all *Glyptotendipes (Heynotendipes)*, *Kiefferulus* and *Acinoretracus* Epler.

Concerning adult males, the feature that has given rise to most of the confusion with *Einfeldia* is the '*Einfeldia*-like' superior volsella with a large, hairy base and a digitiform, bare projection. However, the fact that this volsella configuration does not define any natural grouping (monophylum) was recognised early on (e.g. by Edwards 1929, Kruseman 1933, Freeman 1957). Similar volsellae occur in *Benthalia*, *Chironomus* (subgenera *Chironomus* and *Lobochironomus*), *Conochironomus*, *Einfeldia*, *Glyptotendipes (Heynotendipes)*, *Pelomus*, *Tribelos*, and possibly elsewhere. Even the combination of such superior volsellae with a broad, unconstricted anal point does not define *Einfeldia s. str.*, as it occurs also in other taxa, e.g. in some species of *Chironomus s. str.*

If the convergence argument is turned around, however, some variation added to *Einfeldia* by inclusion of *E. australiensis* may become less significant. This applies especially to the relatively different pupa (see below). For example, patterns of sternite spines as on S II in *E. australiensis* (Fig. 3E) are found in various Chironomini, and never in all species of any genus. Likewise, the segment VIII comb in *E. australiensis* (Fig. 3G) is very different from the combs in other *Einfeldia s. str.*, but the resulting intrageneric variation is no greater than known from, for example, Australian *Dicrotendipes* pupae (Cranston 1996).

Placement of *C. (X.) australiensis* in *Einfeldia*. In the keys to Chironomidae in New South Wales (Cranston 1996), the larva of *C. (X.) australiensis* runs to *Dicrotendipes* Kieffer, the pupa to *Kiefferulus* Goetghebuer. Neither generic assignment looked plausible (see below), and *Einfeldia* Kieffer was not included in that work; thus, '*Xenochironomus australiensis*' was left 'not ... generically placed' (*op. cit.*: 261). In Yamamoto *et al.* (2015), *C. australiensis* seemed to fit reasonably in the '*pagana*-group' of *Einfeldia sensu lato*. Additional comparable references are unavailable for Old-World regions in the southern hemisphere, and there are as yet no molecular data on *C. australiensis*. Therefore, we proceeded to consulting keys and diagnoses to genera in the Holarctic region: Pinder & Reiss (1983) and Epler *et al.* (2013) for the larva, Pinder & Reiss (1986) for the pupa, and Cranston *et al.* (1989) for the adult male. In these works the larva and adult male of *E. australiensis* run readily to *Einfeldia* Kieffer, whereas the pupa fails all Holarctic genus diagnoses in at least one key character each. Individual life-stage features potentially relevant here are the following [respective Holarctic diagnosis condition in square parentheses].

Notable differences between the larva of *E. australiensis* and the diagnosis for *Einfeldia* in Epler *et al.* (2013) are the completely fragmented lateral sclerites anterodorsally on the head capsule [lateral sclerite 3 complete], the tripartite pecten epipharyngis without dorsal spinules [pecten either simple without dorsal spinules or tripartite with spinules], and the evenly decreasing lateral mentum teeth [first and second laterals closely adjacent]. We do not consider any discrepancy as significant enough to rule out inclusion of *E. australiensis* in *Einfeldia*.

Concerning the adult male, the differences are the slightly indicated scutal tubercle [tubercle absent], the anal tergite bands being moderately developed [weak], the gonostylus with relatively little widening in its mid-section [with drastic narrowing between the very wide proximal and the distal section], and the superior volsella with only

very small dorso-medial projection [distal part much larger, digitiform]. For some of these differences, the significance cannot be evaluated conclusively without thorough revision of all known *Einfeldia*. At present it seems reasonable to include *E. australiensis* in *Einfeldia*.

In contrast to the larva and adult male of *E. australiensis*, the pupal morphology falls considerably outside the current diagnosis for *Einfeldia s. str.* (which is nearly congruent with *E.* 'species group A' Pinder & Reiss 1986). Note here that the lectotype exuviae of *E. pectoralis* shows only one major difference from the diagnosis, as abdominal segment I has a pair of anterolateral protrusions similar to the posterior ones on segment II ('pedes spurii'), whereas no evidence of such extensions was given in Pinder & Reiss (1986: 320, fig. 10.19F); in *E. australiensis* they are absent. Potentially significant differences between the Australian pupae and those in Holarctic *Einfeldia s. str.* [data in square parentheses] are as follows. Tracheal plate in basal ring double, its two parts separated by a narrow gap (Fig. 3B) [plate often slightly constricted but simple, its two tracheal bundles in a field with common outline]; abdominal tergite I with posterior transverse shagreen, T II armament similar to that on T III–VI, T VIII unarmed [tergite I unarmed, T II armament distinctly weaker than on T III–VI, T VIII with paired shagreen patches]; sternite II with armament pattern including some strong, translucent spines (Fig. 3E) [S II without such pattern]; segments III and IV with 3 lateral setae each, [segment IV with 4 ls, the posterior-most often on conjunctive IV/V]; segment VIII with 4 lateral taeniae and posterolateral comb of strong, longitudinally serial teeth (Fig. 3G) [5 lateral taeniae, and comb of small, partially overlapping points in cluster posterior to taenia 5].

Despite these differences, and others considered as comparatively minor, the overall pupal morphology in *E. australiensis* remains more compatible with *Einfeldia* than with any other established genus; see the section on 'Other genera considered' below. Although *E. australiensis* differs from Holarctic *Einfeldia* pupae as detailed above, several other features support placing the species among the forms keyed by Pinder & Reiss (1986) in couplet 53, *i.e.* *Einfeldia* 'species group A' and large parts of *Chironomus* in the wide sense of Epler *et al.* (2013). Some features shared in this taxon grouping are the plumose thoracic horn with 2 (rather than 3) bundles of tracheae passing the tracheal plate, the form of the cephalic tubercles, and the presence of pedes spurii B on segment II and pedes spurii A on IV. Indications of affinity especially to *Einfeldia* may be found in the double prealar tubercles (Fig. 3C), which Peter Langton (pers. comm. 2016) has observed also in *E. pagana*, and in the general pattern of tergite armament that includes posteromedian spines darkened and stronger relative to those more laterally and anteriorly (Fig. 3D, F), and that leaves small bare areas from which setae $D_{(3)4-5}$ arise.

Other genera considered. Some *Demeijerea* larvae resemble those in *Einfeldia s. str.*, for example in having a fused frontoclypeus that includes a large fenestra, but their S1 setae are weakly plumose at most (Epler *et al.* 2013: fig. 10.14F, G). *Demeijerea* pupae have diagnostic abdominal tergite armament including special plates or 'point patches' (Pinder & Reiss 1986: fig. 10.15).

The larvae of *Dicrotendipes lobiger* show dorsal head sclerites very similar to those in *E. australiensis*, including the 'scalloping' around the frontoclypeal fenestra (Epler *et al.* 2013: fig. 10.16C). However, in *D. lobiger* the pecten epipharyngis consists of a single plate with few teeth, and the ventromental plate has significantly different dimensions.

Larvae in *Glyptotendipes (Heynotendipes)* have the frons and clypeus separate, mandibles without dorsal teeth and with only 2 inner ones (Epler *et al.* 2013: fig. 10.26), and no ventral tubules on the postabdomen. The pupae have diagnostic abdominal tergite armament including special plates or 'point patches' (Pinder & Reiss 1986: figs. 10.25–10.27).

Kiefferulus larvae have the clypeus separate and the premandibles multitoothed. Some *Kiefferulus* pupae show similar abdominal tergite armament and segment VIII combs while also lacking conjunctive setae, but differ by the presence of armed anterolateral tubercles on sternum I and by possession of dorsal armament on at least one conjunctive.

Acinoretracus larvae have the clypeus either free (*A. multispinosus* (Freeman)) or fused (*A. penicillatus* (Freeman)); S1 is plumose on inner side only; premandibles are multitoothed; the pecten epipharyngis is a single multitoothed plate and the ventromental plates have a unique sculpturing. The pupa has low rounded cephalic tubercles; there is an anteriorly directed flange-like lobe on the base of the thoracic horn and pedes spurii B are lacking (Epler *et al.* 1998).

Xenochironomus larvae have head capsules with distinctive adaptations to their lives as miners in sponges. All *Xenochironomus* pupae lack posterolateral combs on abdominal segment VIII, and have characteristic tergite armament patterns (Pinder & Reiss 1986, Cranston 1996).

Concluding remarks. Edwards (1929) joined several of his contemporaries in stating the critical importance of the chironomid immature stages, noting that “almost nothing has been done here [in Britain] by way of rearing these insects, and until this omission has been remedied no true idea of their relationships nor of specific limits can be obtained, nor can the nomenclature be correlated fully with that employed by continental workers.” Although the lectotype now presents a handle on pupal morphology in *E. pectoralis*, some taxonomic uncertainty lingers as long as reliably associated specimens of the species' other life stages remain to be found and/or analysed. Another, even greater necessity will be a comprehensive revision of all remaining species of *Einfeldia*, but this is beyond the scope of the present work. As things stand, however, we are confident that modifications to previous diagnoses of *Einfeldia* resulting from inclusion of our Australian specimens are reasonable, whereas erecting a monotypic new genus for *C. (X.) australiensis* would be significantly less informative.

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