Received: 7 August 2018 | Revised: 23 October 2018 | Accepted: 30 October 2018

DOI: 10.1111/zsc.12331

ORIGINAL ARTICLE

WILEY Zoologica Scripta

A new surface gliding species of Chironomidae: An independent invasion of marine environments and its evolutionary implications

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Funding information
National Natural Science Foundation of China, Grant/Award Number: 31301908 and 31672264; Zhejiang Provincial Natural Science Foundation of China, Grant/Award Number: LY17C040001

[Version of Record, published online on 13 December 2018; http://zoobank.org/6AE5D4A6-B76F-42D7-B03A-495CB45FA4E4]

Abstract

Insects have invaded marine habitats only rarely and secondarily. Recently, we discovered a flightless dipteran species skating rapidly on the surface of seawater ponds at the Pacific coast of eastern China. Morphological analyses initially suggested an isolated position of the non-biting midge, suggesting the erection of a new genus within Chironomini (Diptera: Chironomidae). However, an analysis of molecular data revealed that the marine species is in fact nested within the species-rich genus *Dicrotendipes*. The apparent conflict between molecular and morphological data can be easily explained. It is likely that the new species has evolved a series of autapomorphic adaptations. These traits clearly distinguish the taxon from other species of the genus but do not justify the erection of a new supraspecific taxon, which would render *Dicrotendipes* paraphyletic. The switch to marine environments was likely a trigger for various morphological modifications resulting from increased selective pressure. Molecular data suggest that the potential speciation event occurred around 19–29 Ma, linked with a migration from freshwater to seawater ponds along the Pacific Ocean. Considering the results of our analysis, we place the flightless marine skater in the genus *Dicrotendipes*. All life stages of *Dicrotendipes sinicus* Qi & Lin sp. n. are described and illustrated, associated with larvae obtained by rearing or confirmed through association with DNA barcodes. The biology and ecology of the species are outlined based on collection data and in situ observations. Evolutionary patterns linked with repeated invasions of marine habitats are discussed.

KEYWORDS

adaptation, flightless, marine, phylogeny, skate

1 | INTRODUCTION

Despite the recently documented decline in insect diversity (Costello, May, & Stork, 2013) and biomass (Hallmann et al., 2017), insects are still by far the most diverse group of animals (Stork, McBroom, Gely, & Hamilton, 2015), often occurring in immense densities. The group can be found in a multitude of terrestrial and freshwater habitats and play an integrative role in these environments, forming an essential part of food chains. However, despite their remarkable ecological versatility and pancrustacean ancestry (Misof et al., 2014), only few insects inhabit marine habitats, and only peripherally and secondarily

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(e.g., Grimaldi & Engel, 2005). A switch to the marine environment is apparently a great challenge for the physiology and locomotor apparatus of insects. More than 30,000 species occur in different lentic or lotic freshwater habitats (Cheng, 1976). In contrast to this, only a few thousand live in marine tide pools, seawater ponds, or in exceptional cases also the open sea.

Few insect species have evolved morphological and physiological adaptations to a glide on the water surface (Cheng, 1976). They are arguably most pronounced in the water skaters, for example, the marine *Halobates* Eschscholtz, 1822 (Heteroptera) and in a number of non-biting midges such as the marine *Pontomyia* Edwards, 1926 (Diptera). Species of these taxa have strongly developed, elongated forelegs, reduced wings, and a hydrophobic body surface.

The Chironomidae (non-biting midges) is a dipteran family with more than 6,000 described species worldwide (P. Ashe, pers. comm.). The larvae usually develop in freshwater, but a limited number of marine and terrestrial species occur. These are taxonomically widely distributed among 12 genera of the three subfamilies Chironominae, Orthocladiinae, and Telmatogetoninae (Armitage, Cranston, & Pinder, 1995). A number of marine chironomids have been recorded from the Pacific Ocean, for instance the majority of *Clunio* Haliday, 1855, *Pontomyia* Edwards, *Telmatogeton* Schiner, 1866, and *Thalassomya* Schiner, 1856 (Hashimoto, 1976), and one species of *Tanytarsus* van der Wulp, 1874 (Sugimaru, Kawai, & Imabayashi, 2008).

Evolutionary and phylogenetic links between freshwater and marine chironomid taxa are insufficiently understood at present. It remains unclear why species of some clades can occur in extremely different habitats and which forces drive allopatric speciation in natural populations. Consequently, we used a newly discovered marine species to investigate these phenomena. A combination of morphological, molecular, developmental (postembryonic), and ecological data may yield insights into phylogenetic, evolutionary, and biogeographic patterns among freshwater and marine populations.

Dicrotendipes sinicus Qi & Lin sp. n. is described, and its systematics placement and taxonomic status are discussed based on morphological and molecular data. The biology and ecology of the hitherto unknown marine species are outlined based on collection data and in situ observations.

2 | MATERIAL AND METHODS

2.1 | Taxon sampling

The marine chironomids used in this study were collected from the seawater ponds of Xishan Island, Zhoushan and Sanmen, Taizhou, Zhejiang, China (Figure 1) in the early summer and autumn during 2012–2016. Xishan Island located south-east of Zhoushan, Zhejiang, China, belongs to subtropical climate zone and covers an area of 0.37 km^2. The

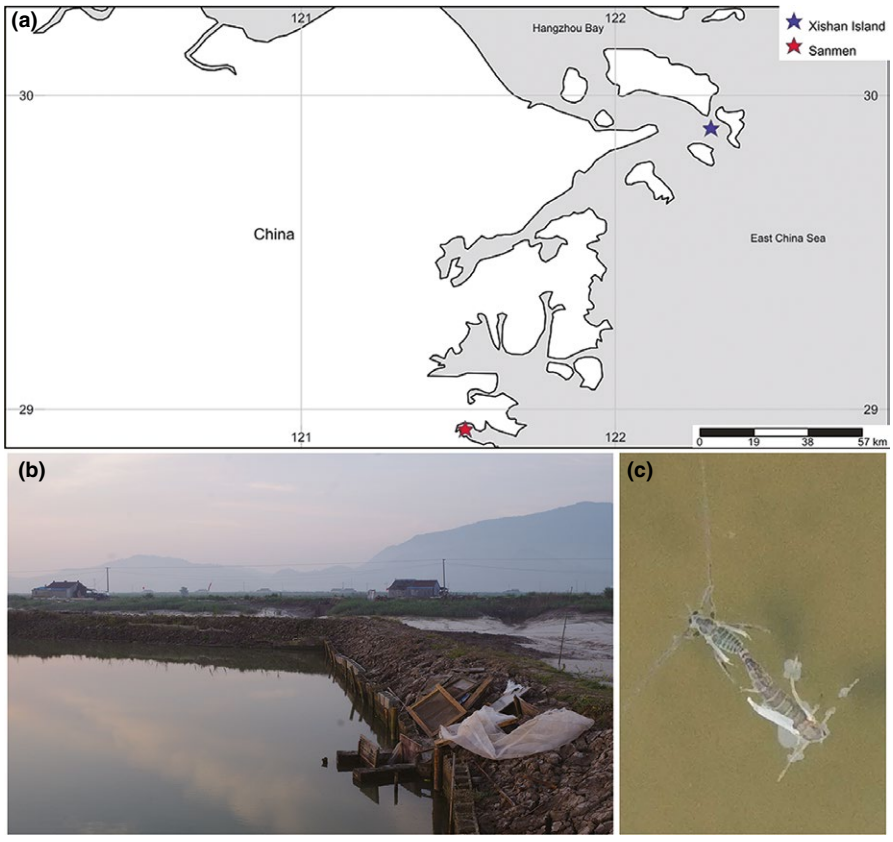

FIGURE 1 Sampling locality of *Dicrotendipes sinicus* Qi & Lin sp. n. (a) Sampling locality; (b) Seawater pond in Sanmen, Zhejiang, China; (c) Adults mating on a seawater pond

other sampling locality at Sanmen is a coastal seawater breeding pond for mud crabs. Additionally, one species of *Axarus* Roback, 1980, nine of *Dicrotendipes* Kieffer, 1913, one of *Fleuria* Kieffer, 1924 and one of *Kiefferulus* Goetghebuer, 1922 from China have been included in the sampling for an updated molecular phylogeny of Chironomini. The molecular data for the remaining taxa were retrieved from a previously published study (Cranston, Hardy, & Morse, 2012).

2.2 | Morphological study

The morphological nomenclature follows the standard in chironomid taxonomy (Sæther, 1980) except for the "filamentous setae (LS)" in pupal exuviae (here called "taeniae") (Langton, 1994). The examined specimens were slide-mounted in Euparal, following the procedures outlined by Sæther (1969). Measurements are given as ranges followed by the mean, when four or more specimens are measured, followed by the number of specimens measured. Specimens were identified morphologically using relevant taxonomic revisions and species descriptions (Albu, 1980; Cranston, Dillon, Pinder, & Reiss, 1989; Epler, 1988; Lin & Wang, 2012; Niitsuma, 1995; Qi, Lin, & Wang, 2012).

2.3 | Digital microscopy

Digital photographs of the unknown marine species were taken with a resolution of 300 dpi with a Leica DFC420 camera mounted on a Leica DM6000 B compound microscope using bright field or Nomarski DIC and the software Leica Application Suite at the NTNU University Museum, NTNU (Trondheim, Norway). The adult males were illustrated with a resolution of 600 dpi in Adobe Photoshop and Illustrator (Adobe Inc., California, USA).

2.4 | Scanning electron microscopy

Specimens were cleaned with liquid soap, dehydrated in an ethanol series, dried with acetone, mounted on a needle on a rotatable specimen holder (Pohl, 2010), and coated with gold using an EmiTech K500 sputter coater. SEM micrographs were taken with a Philips XL 30 ESEM equipped with Scandium software at Institut für Spezielle Zoologie und Evolutionsbiologie of the FSU, Jena, Germany.

2.5 | DNA extraction, PCR amplification, and sequencing

Adult specimens were preserved in 85% ethanol, immatures in 96% ethanol, and stored dark at 4°C before morphological and molecular analyses. Genomic DNA of most specimens was extracted from the thorax and head of adults, and abdominal segments of larvae using QIAGEN DNeasy® Blood and Tissue Kit at the Department of Natural History, NTNU University Museum, Trondheim, Norway and College of Life Sciences, Nankai University, Tianjin, China following the standard protocol. The lysis was done overnight and the final elution volume was 100 µl. After DNA extraction, the cleared exoskeleton was mounted in Euparal on microscopic slides with the corresponding wings, legs, and antennae.

Fragments of one mitochondrial gene (COI-3P), two ribosomal genes (18S and 28S), and two sections of a nuclear protein-coding gene (CADI and CADIV) were amplified to update the molecular dataset used to explore the phylogeny of Chironomini. The specific primers (Supporting Information Table S1) used to amplify the five regions are the same as in Cranston et al. (2012), and the universal primers LCO1490 and HCO2198 (Folmer, Black, Hoeh, Lutz, & Vrijenhoek, 1994) were used for the standard COI barcode sequences.

PCR amplification of two segments of COI was carried out in 25 µl reactions using 2.5 µl 10× Takara ExTaq buffer (CL), 2 µl 2.5 mM dNTP mix, 2 µl 25 mM $MgCl_2$, 0.2 µl Takara Ex Taq HS, 1 µl 10 µM of each primer, 2 µl template DNA and 14.3 µl ddH_2O. Amplification cycles were performed on a Bio-Rad C1000 Thermal Cycler (Bio-Rad, CA, USA) and followed a program with an initial denaturation step of 95°C for 5 min, then followed by 34 cycles of 94°C for 30 s, 51°C for 30 s, 72°C for 1 min and 1 final extension at 72°C for 3 min. CADI and CADIV were amplified with a touchdown program: initial denaturation step of 98°C for 10 s, then 94°C for 1 min followed by five cycles of 94°C for 30 s, 52°C for 30 s, 72°C for 2 min and seven cycles of 94°C for 30 s, 51°C for 1 min, 72°C for 2 min and 37 cycles of 94°C for 30 s, 45°C for 20 s, 72°C for 2 min 30 s and one final extension at 72°C for 3 min. Fragments of 18S and 28S were amplified with an initial denaturation step of 98°C for 10 s, then 95°C for 3 min followed with subsequent cycling as follows: In each cycle, denaturation was performed at 72°C for 1 min; the annealing temperature of the reaction was decreased by 2°C every sixth cycle from 57°C to a touchdown at 47°C, a final additional elongation step at 72°C for 10 min. PCR products were visualized on a 1% agarose gel, purified using Illustra ExoProStar 1-Step and shipped to MWG Eurofins or BGI for bidirectional sequencing.

DNA sequences were edited with Sequencher 4.8 (Gene Codes Corp.). In cases of ambiguity of base calls, we used the appropriate IUPAC code, but replaced the ambiguity symbol ''N" with ''?" in the data matrices. Protein-coding genes were aligned on amino acids using Muscle (Edgar, 2004) in MEGA6 (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013). Introns in CAD were recognized by the GT-AG rule (Rogers & Wall, 1980). The codons were aligned after removing introns. Ambiguous regions for 18S and 28S rDNA were excluded with GBlocks v0.91b using default setting except allowing gap positions within the final blocks

(Castresana, 2000; Talavera & Castresana, 2007). The aligned sequences are available in Supporting Information Appendix S1.

2.6 | Phylogenetic analyses of molecular data

All selected genes were concatenated using SequenceMatrix v1.7.8 (Vaidya, Lohman, & Meier, 2011) to reconstruct phylogenetic relationships of available species of Chironomini. The final dataset consists of 3,711 bp in a matrix of 50 terminal taxa (Supporting Information Table S2). Phylogenetic analyses used the partition strategies and models of sequence evolution were determined under the "greedy search" algorithm based on the Bayesian information criterion in PartitionFinder V1.1.1 (Lanfear, Calcott, Ho, & Guindon, 2012). Branch lengths were unlinked to allow the program to estimate them independently for each subset. The best optimal models were HKY + G for the 3rd codon for COI-3P, SYM + G + I for the 3rd codon for CAD gene, and GTR + G + I for the 18S, 28S and the first two codons for all protein-coding genes. We used a maximum likelihood (ML) phylogenetic analysis on concatenated nuclear gene dataset with RAxML8.1.2 (Stamatakis, 2006, 2014) using raxmlGUI v1.5b1 (Silvestro & Michalak, 2012), with unlinked partitions as selected by PartitionFinder. We used 1,000 bootstrap replicates in a rapid bootstrap analysis, searching for the best scoring ML tree with the model GTR + G + I. We implemented Bayesian inference in MrBayes v3.2.6 (Ronquist et al., 2012). In the Bayesian analyses, datasets were partitioned by gene and codon for the protein-coding genes and by gene for the non-coding gene, Markov Chain Monte Carlo (MCMC) iterations were run with four chains on two runs for 10 million generations, sampled every 1,000 generations with a burn-in of 0.25 with the best fitting models selected by PartitionFinder. Convergence among the runs was monitored using Tracer v1.6 (Rambaut, Suchard, Xie, & Drummond, 2014), where we discarded first 25% trees as burn-in. The average standard deviation of split frequencies was 0.004.

Phylogenetic divergence times were estimated using BEAST V1.8.2 (Drummond, Suchard, Xie, & Rambaut, 2012) based on the same partitioned dataset. Similar to Cranston et al. (2012), a separate HKY + G model was applied to each partition. Empirical experience has shown that analysis using the HKY + G model reaches convergence faster than the GTR + G model without significant differences in the results. The uncorrelated lognormal relaxed-clock model for among-lineage rate variation was used in conjunction with a Yule speciation model. A lognormal (initial value = 1.0, Log[mean] = 0.0, Log[*SD*] = 1.0, offset = 0) prior was applied to the ucld. mean parameter. Based on previous dating analyses by Cranston et al. (2012), we set the root height with a normal prior (initial = 132.9 Ma, mean = 149.8 Ma, *SD* =8 Ma), and outgroup (*Shangomyia* + *Xiaomyia*) with a normal prior (initial = 61.9, mean = 86.4, *SD* = 10.5). In addition, the inter node including all sampled *Dicrotendipes* species was calibrated with a normal prior (initial = 50 Ma, mean = 51 Ma, *SD* = 0.4 Ma) base on an Indian Cambay stem fossil of *Dicrotendipes* dated to 50–52 Ma (V. Baranov pers. comm.). Based on the phylogenetic results from the MrBayes runs, the BEAST analysis was run with several topological constraints on monophyletic taxa, that is taxon set 1 (all sampled *Polypedilum* species), taxon set 2 (*Xiaomyia* + *Shangomyia*), taxon set 3 (*Fleuria* + *Chironomus* + *Pagastiella*), taxon set 4 (all sampled *Dicrotendipes* species including the unknown marine species), taxon set 5 (*Polypedilum* + *Chironomini* sp. 1 + *Endochironomus* + *Endotribelos* + *Stictochironomus* + *Xylochironomus* + *Harrisius* + *Stenochironomus* + *Xestochironomus*), taxon set 6 (*Imparipecten* + *Nilothauma*). The MCMC was run for 60 million generations, sampling trees every 10,000 generations after discarding samples from the first 6 million generations. Tracer v1.6 was used to examine the BEAST log file and ESSs for each parameter which was all >200. The maximum clade credibility tree with median heights was generated using TreeAnnotator v1.8.2 with 6 million states as burn-in.

3 | RESULTS AND DISCUSSION

3.1 | Morphology

Using presently available keys to adult males, larvae and pupae of Chironominae (Cranston et al., 1989; Pinder & Reiss, 1983, 1986) and a key to genera of marine chironomids (Hashimoto, 1976), the species described here is identified as a representative of the *Chironomus* generic complex in the tribe Chironomini of the subfamily Chironominae (Diptera: Chironomidae). However, it cannot be assigned to any described genus of the tribe. Using the key to adult females of Chironomini (Sæther, 1977), the marine species appears close to *Chironomus* (*Halliella*), which is a synonym of *Chironomus* (*Baeotendipes*) (Epler, Ekrem, & Cranston, 2013), by having a reduced palp, six-segmented antenna, a developed antepronotum (in adult females) and a similar saline habitat of larvae (Cranston et al., 1989). After adding morphological characters of adult males and immatures stages, the marine species does not key to any known species of this subgenus, which occur in entirely different geographic regions: *Chironomus* (*B.*) *noctivagus* (Kieffer, 1911) from Europe and North Africa, and *Chironomus* (*B.*) *ovazzai* (Freeman, 1957) from Africa (Freeman, 1957).

Using morphological features of all life stages, the placement of the marine species remains ambiguous. It

TABLE 1 Comparison of selected morphological features of the new marine species, *Chironomus* (*Baeotendipes*), *Dicrotendipes,* and *Kiefferulus*

	Marine species (*Dicrotendipes sinicus* sp. n.)	***Chironomus* (*Baeotendipes*)**	***Dicrotendipes***	***Kiefferulus***
Adult male				
Dorsomedial parallel-sided extension of eye	Absent	Strong	Strong	Strong
Flagellomeres	10	11	11	11
Palp	Shortened, 5-segments	Well-developed, 5-segments	Well-developed, 5-segments	Well-developed, 5-segments
Antepronotal lobes	Dorsally separated	Fused at notch	Narrowed and weakly notched medially	Dorsally separated
Acrostichals	Absent	Present	Present	Present
Wing	Shortened, oar-shaped, with tuft of long setae apically	Well-developed	Well-developed	Well-developed
Squama	Glabrous	Setose	Setose	Setose
Fore leg	Strongly elongated	Normal size	Normal size	Normal size
Mid and hind legs	Reduced	Reduced	Developed	Developed
Combs of mid and hind tibiae	Widely separated, each bearing one stout spur	Very narrowly separated, each bearing 1–4 spurs	Closely approximated, each bearing longspur	Narrowly separated, slightly raised and each with one spur
Tergites VII–IX	Inverse	Not inverse	Not inverse	Not inverse
Superior volsella	Absent	Present	Present	Present
Median volsella	Present	Absent	Usually absent, present only in two australian species	Absent
Length of inferior volsella	Extending beyond gonostylus	Not reaching apex of gonostylus	Not reaching apex of gonostylus	Not extending beyond apex of gonostylus
Gonostylus	Moveable, with a few teeth distally	Fixed, without teeth	Fixed, without teeth	Fixed, without teeth
Adult female				
Flagellomeres	6	5	5	5
Palpomeres	3	2–4	5	5
Wing	Shortened as in adult males or well-developed	Well-developed	Well-developed	Well-developed
Combs of mid and hind tibiae	Widely separated; mid-tibia with two stout spurs; hind tibia with only one spur.	Very narrowly separated, each bearing 1–4 spurs	Closely approximated, each bearing longspur	Narrowly separated, slightly raised and each with one spur
Pupa				
Pedes spurii B	Absent	Present on segment II	Present on segment II, sometimes indistinct	Absent
Cephalic tubercles	Present	Present	Present	Absent
Basal ring	With two separated tracheal marks	With two fused tracheal marks	With two fused tracheal marks	With two fused tracheal marks
LT setae on segment VIII	4	5	4–5	5
Anal comb	Dark brown, with strong and short spines	Slender	Slender, or with large and small spines	With long slender teeth in single row
Larva				
Premandible	4 teeth	5 teeth	3 teeth	5–7 teeth
Ventral tubules	Absent	Absent	Usually absent or one pair only in two species	One pair

appears most closely related to the subgenus *Chironomus* (*Baeotendipes*) and the genera *Dicrotendipes* and *Kiefferulus*. It differs from these taxa by the following morphological characters as shown in Table 1.

3.2 | DNA barcodes

Six COI DNA barcodes of adults and a larva cluster into the same BIN (BOLD:ACX3644), with maximum intraspecific

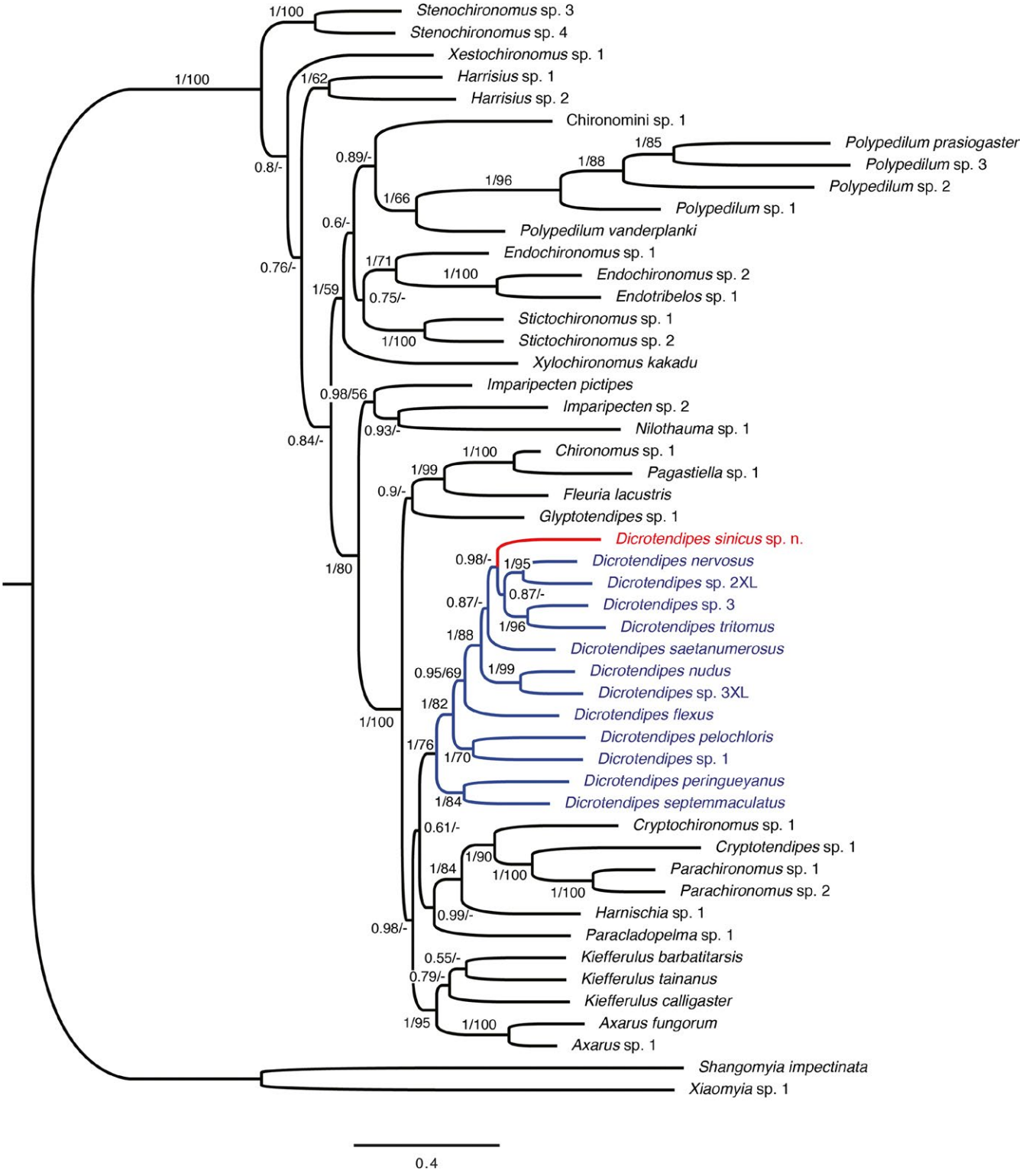

FIGURE 2 Bayesian tree of the tribe Chironomini showing the position of *Dicrotendipes sinicus* Qi & Lin sp. n. Nodes are labelled with posterior probabilities followed by bootstrap support from maximum likelihood analyses. Values below 50 are marked with "–." Scale bar reflects expected changes per site

pairwise genetic distance of 0.16%, and 9.6% divergence to the nearest BIN (BOLD:AAP2997). Members of the nearest BIN are unidentified in BOLD, but photographs of adult males show a normally developed, fully winged species of Chironomini. Using DNA barcodes, adult females with two different wing morphologies were associated with adult males and larvae.

3.3 | Molecular phylogenetic analyses

We ran initial phylogenetic analyses based on published data from Cranston et al. (2012) and found that the marine species is nested within *Dicrotendipes* in both ML and Bayesian inference trees (Figure 2). As it was not possible to examine the *Dicrotendipes* vouchers used in Cranston et al. (2012), we added nine other species of the genus from China to explore the systematic position of the new marine chironomid. In contrast to the placement suggested by morphology-based keys (see above), our molecular phylogenetic trees (Figure 2) confirmed that the marine species is nested within *Dicrotendipes*. *Dicrotendipes* is a species-rich genus, including more than 100 species worldwide, and at least the larvae of four species develop in brackish water (Epler, 1988).

3.4 | Biology

3.4.1 | Life history

Emerging and mating occurred in the early morning and dusk.

3.4.2 | Reproduction

In general, the adults emerge in early summer, especially when the atmospheric pressure is low (e.g., before approaching typhoon). After emergence, the adults skate rapidly on the surface of the marine habitat, seeking mating partners. Although the females are larger and appear more robust than males, the males actively catch the females. The rotated male abdomen contacts and connects with the genitalia of the female. The couple continues skating on the surface (Supporting Information Video S1).

3.4.3 | Seasonality

Dicrotendipes sinicus sp. n. appears mostly in ponds where the seawater temperature is 20–25°C. The larval density rapidly increases from the end of April to early June, and from middle to end of September. By early summer and autumn, the larvae practically disappear and do not reoccur until the next year. There is a simple explanation for the annual rapid growth of the population during early summer: fishermen pour numerous eggs of poultry in the seawater ponds as feed for small crabs and shrimps. Thus, there are enough nutrients to support strong algal growth giving larvae of *D. sinicus* sp. n. sufficient food resources. In combination with suitable temperatures, this supports rapid development of the immatures.

3.4.4 | Diet

The digestive tract of larvae of *D. sinicus* sp. n. contained fragments of algal debris, diatoms and one chironomid wing.

3.4.5 | Predators

We observed that crabs (e.g., *Scylla serrata* (Forsskål, 1775) and *Portunus trituberculatus* (Miers, 1876)) consumed chironomid larvae. As the population and biomass of crabs and shrimps grew, *D. sinicus* sp. n. disappeared. We think this was caused by predation by the crustaceans over a period of several weeks. The larvae can thus be considered as an important food item for marine crabs, shrimps, and fishes in seawater pond aquaculture.

3.5 | Ecology

3.5.1 | Special adaptations

Almost all chironomids are flyers, and most species use swarming in the context of mating. However, the flying capacity is lost in males and in parts of the female population of the marine species. This is arguably partly compensated for by the ability to skate on the water surface. Adults of *D. sinicus* sp. n. are only moving rapidly on the water to meet and cling to mating partners. The facultative loss of the flight capacity may be an adaptation to strong sea breezes. The oar-shaped wings (Figure 3) probably create the main propulsive force for surface skating, functioning such as "propellers of an aeroplane" (Hashimoto, 1976 [*Clunio*]; Neumann, 1976). The well-developed halteres and the very long forelegs may also play a role in the skating behavior. The forelegs are probably primarily used to detect and grasp mating partners and are of minor importance for the actual movement on the water surface. The setae on large parts of the body surface, especially on the apical wing region, the thorax including the legs, and on the abdomen, make these body parts hydrophobic (Figure 4).

3.5.2 | Habitat requirements

Currently, *D. sinicus* sp. n. is known from seawater ponds where the salinity is about 26–29‰ and the temperature 20–25°C. It is likely that the new species predominantly lives in adjacent areas of open sea as fishermen clean the seawater ponds every year using hydrated lime [$Ca(OH)_2$] and then pump fresh seawater into the ponds.

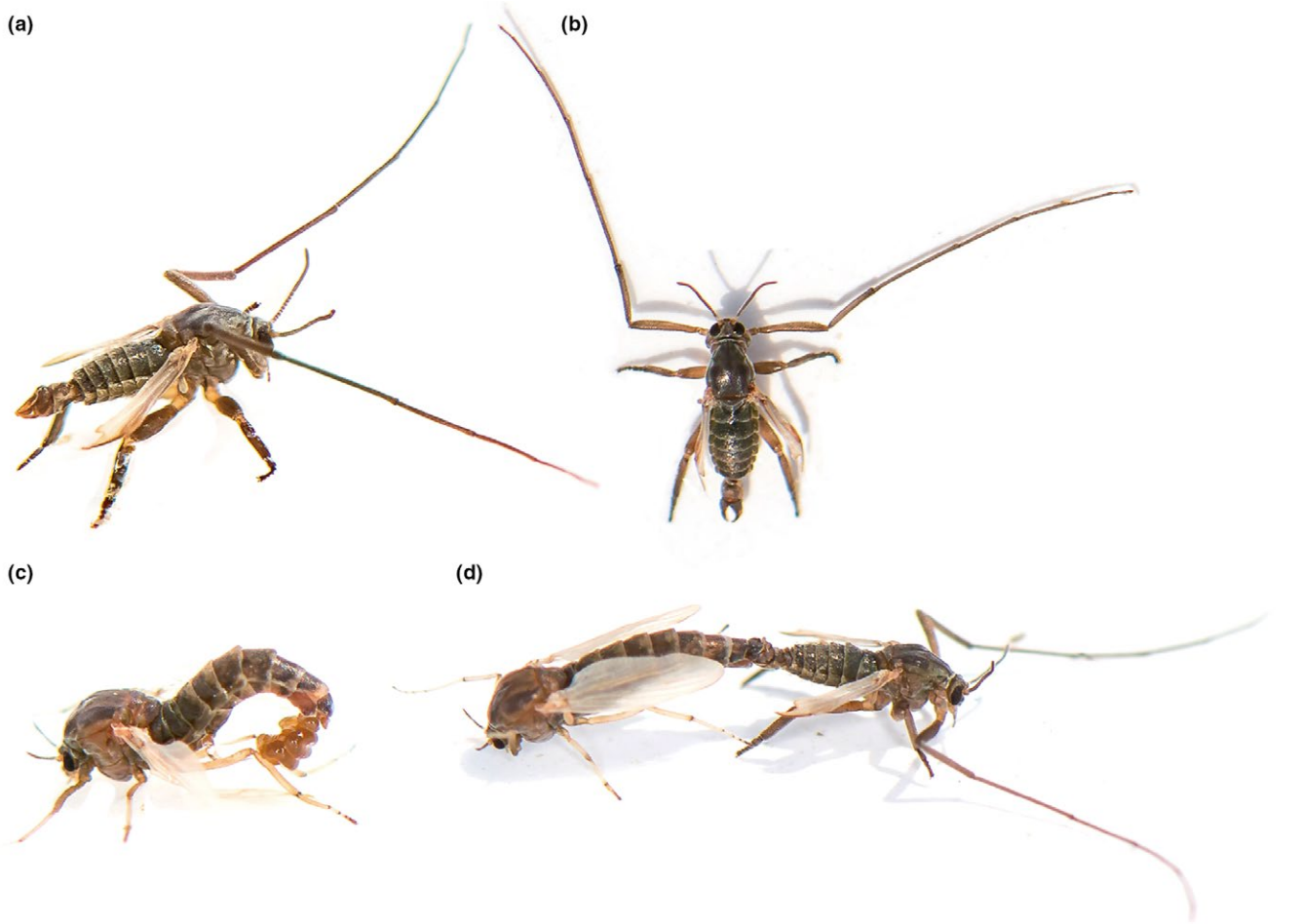

FIGURE 3 Live adults of *Dicrotendipes sinicus* Qi & Lin sp. n. photographed on the water surface. (a,b) Adult male; (c) Adult female laying eggs; (d) Adults mating

3.5.3 | Distribution

At present, the marine species is found only at the southern Chinese coastal region close to Shanghai.

3.6 | Evolutionary considerations

Insects have invaded marine environments only secondarily and peripherally, with only very a few species of water striders of the genus *Halobates* also occurring in the open ocean (e.g., Cheng, 1976). Aside from some true bugs (especially Gerridae), few species of Coleoptera (in Hydraenidae and Staphylinidae) and members of one family of Trichoptera, different groups of Diptera have evolved various adaptations to marine habitats, especially in the larval stages (Cheng, 1976). This includes saltmarsh mosquitos (Culicidae) (O'Meara, 1976), biting midges of mangrove swamps and saltmarshes (Linley, 1976), seaweed flies (Coelopidae) (Dobson, 1976) and intertidal horse flies and deer flies (Tabanidae) (Axtell, 1976). However, Chironomidae are probably the group with the greatest potential to invade marine environments, not only as larvae but some of them also in very specific ways as adults (Neumann, 1976). Hashimoto (1976) listed two genera of Chironominae as marine non-biting midges, five genera of Orthocladiinae (as Clunioninae), and three genera of Telmatogetoninae. The degree of adaptation varies among these groups. The Chironominae *Pontomyia* is arguably best known for its specialized marine lifestyle (Cheng & Collins, 1980; Huang & Cheng, 2011), skating on the water surface as males, with shortened oar-like wings, elongated halteres, strongly elongated forelegs, shortened and robust middle and hind legs, and a fully and permanently rotated hypopygium. Even though *D. sinicus* sp. n. is not a close relative of *Pontomyia* and belongs to a different tribe, the males share a remarkable array of features with those of this genus, characteristics which are absent in other species of *Dicrotendipes*. Like males of *Pontomyia* and *Clunio* (Neumann, 1976) (and the freshwater Chironominae genus *Corynocera*) adults of the new species skate on the water surface. This is made possible by the shortened and stout middle and hind legs with modified distal parts (Figure 3, Supporting Information Figure S3), where especially the pulvilli are set

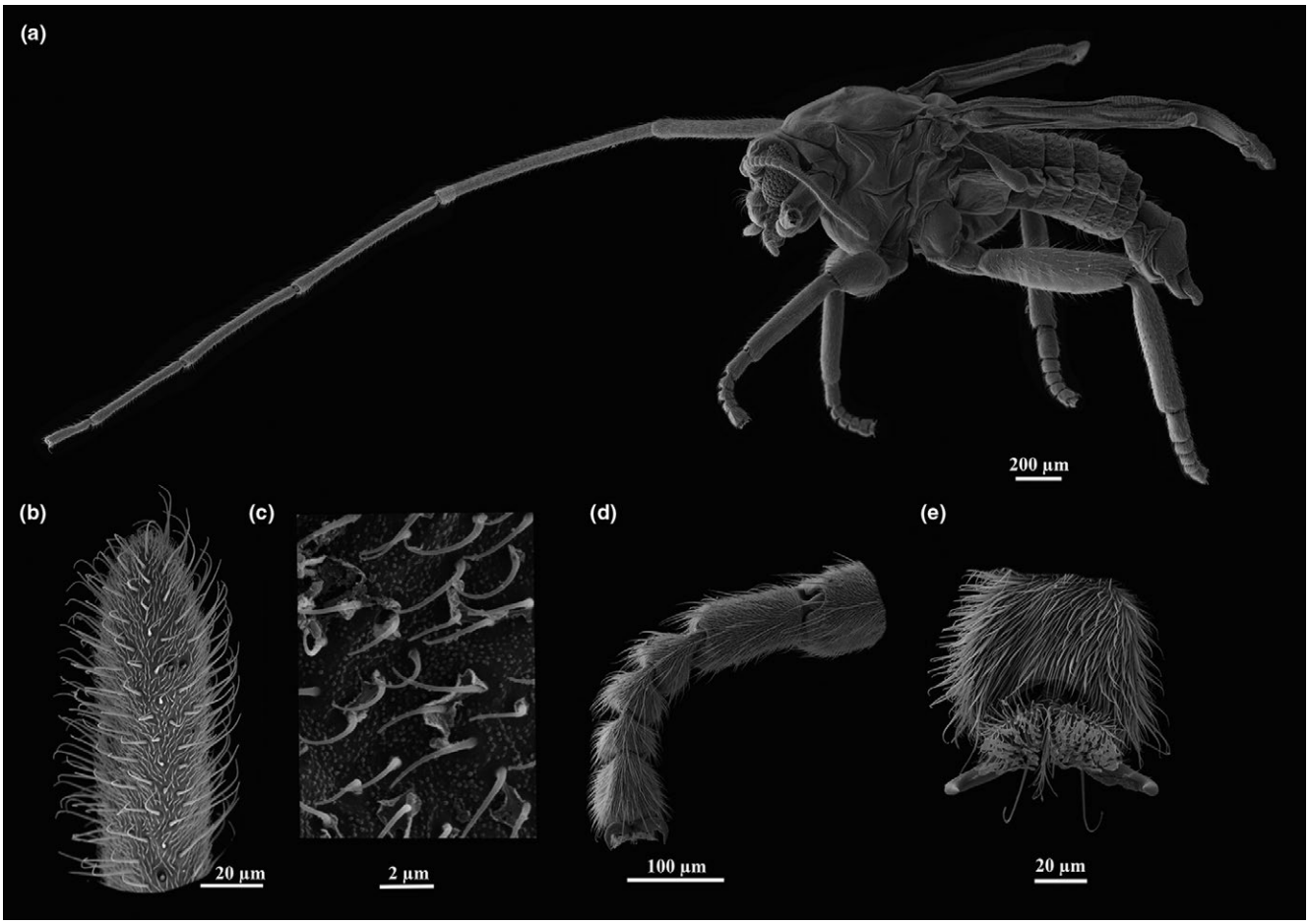

FIGURE 4 SEM images of adult male of *Dicrotendipes sinicus* Qi & Lin sp. n. (a) Body in lateral view, 74× magnification; (b) Ultimate flagellomere of with a few subapical sensilla, 951× magnification; (c) Thorax surface, 8,551× magnification; (d) Tarsomeres of mid-leg, 301× magnification; (e) claw of mid-leg, 851× magnification

with different types of tenent hairs. The fore wings are also modified into oar-like structures, equipped with distal tufts of setae and very likely used as propellers (e.g., Hashimoto, 1962; Neumann, 1976). Another derived feature shared with males of *Pontomyia* and *Clunio* is likely linked with the unusual locomotion, a permanent rotation of the hypopygium (Supporting Information Figures S5 and S6). The copulation starts face-to-face and then switches quickly into an end-to-end arrangement, facilitated by the rotated postabdominal segments of the male. Despite very similar adaptations in the males with surface gliding habits, *D. sinicus* sp. n. differs profoundly from *Pontomyia* and *Clunio* in its sexual dimorphism, or better trimorphism. The hind wings of the females of the new species are either normally developed, or they are shortened and oar-like, similar to those of males. The former enable them to disperse efficiently like the fully winged females of related species of the genus, and the latter probably facilitate contact between mating partners and possibly copulation. This differs strongly from *Pontomyia* and *Clunio*, where the females are more or less immobilized and dragged by the males, with largely or completely reduced wings and simplified, shortened legs. In contrast, the legs of the females of the new species well-developed, elongate, and slender.

Phylogenetic reconstructions show clearly that marine habits have evolved several or multiple times independently in Chironomidae, partly with similar adaptations, but partly also with distinctly different modifications. The newly described species with its remarkable set of adaptations underlines the outstanding potential of phenotypic adaptations of the family in the context of marine life habits. It reveals a new independent invasion of the marine environment, which is apparently very challenging for insects physiologically, and also in terms of locomotion and reproduction (e.g. Cheng, 1976).

Apart from evolutionary aspects, *D. sinicus* sp. n. is an interesting case study taxonomically. It is a persistent problem in taxonomy, that supraspecific taxa are uncritically erected for species with conspicuous or accumulated apomorphic features. This is often practiced without testing the monophyly of existing genera (Komarek & Beutel, 2007), and without using molecular data as independent source of evidence. This can result in genera solely based on autapomorphies and leaving behind paraphyletic taxonomic

units. A traditional approach based on morphological characters and identification keys suggests an isolated position of the new marine species or a placement in the subgenus *Baeotendipes* of *Chironomus*. The apparent discrepancies with the existing generic concepts support the erection of a new genus. However, the phylogenetic analyses based on molecular data clearly show that that the species is deeply nested in *Dicrotendipes*, a diverse Chironominae genus hitherto only composed of freshwater or brackish water species. This is also in agreement with characters of the pupae and larvae (P. S. Cranston, pers. comm.).

The seemingly conflicting genetic and morphological data suggest that the switch to the marine environment was a strong trigger for far-reaching morphological transformations, with increased selective pressure, resulting in a distinctly different adult phenotype and locomotor apparatus, and also an unusual sexual trimorphism, with females either with shortened and oar-like wings, or fully capable of flight. The molecular analyses indicate that the speciation event in *Dicrotendipes* took place in the early Miocene or late Oligocene (19–29 Ma, Supporting Information Figure S10), linked with the switch from freshwater to the marine environment. What presently remains elusive is the original habitat of the new species. So far, records are restricted to artificial marine ponds. Therefore, increased collecting efforts in the region should have high priority.

3.7 | Taxonomy

Detailed description of *D. sinicus* Qi & Lin sp. n. is given in the Supplementary Materials (Supporting Information Appendix S2; Supporting Information Figures S1–S9).

4 | CONCLUSION

The new species *D. sinicus* Qi & Lin sp. n. exemplifies the great potential for phenotypic change in the Chironomidae and presents new knowledge of adaptations to a marine environment and water surface mating. Although phylogenetically distantly related, the new species shows morphological similarities to other water skating chironomids, but also distinct autapomorphic features, especially in the females. Our results emphasize the necessity of careful phylogenetic evaluations before erecting new supraspecific taxa based on specific adaptations or accumulated autapomorphies.

ACKNOWLEDGEMENTS

Many thanks are due to Zheng Liang for some of the figures of adult males, and to Si-Pei Liu for helping with critical point drying of specimens for SEM. Financial support from the Zhejiang Provincial Natural Science Foundation of China (LY17C040001) and the National Natural Science Foundation of China (31301908, 31672264) are acknowledged with thanks.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Qi X, Lin X-L, Ekrem T, et al. A new surface gliding species of Chironomidae: An independent invasion of marine environments and its evolutionary implications. *Zool Scr*. 2018;00:1–12. https://doi.org/10.1111/zsc.12331

Dicrotendipes sinicus Qi & Lin **sp. n.** (Figs S1–S9)

http://zoobank.org/00692809-2316-452B-8FC4-3DD126F25656

Type material. Holotype, ♂ & Pe (BDN: J2A19), China: Zhejiang, Zhoushan, Xishan Island, seawater ponds, 29.896°N, 122.305°E, 1 m a.s.l., 5.VI.2012, hand net, leg. X. Qi. Paratypes, China: 9♂♂ (BDN: J2A2, J2A5, J2A6, J2A7, J2A20, J2A26, J2A27, J2A29, J2A33), 1♂ & Pe (BDN: J2A4), 3♀♀ & Pe (BDN. J2A1, J2A28, J2A29), 1P (BDN. J2A22), 2Pe (BDN: J2A6, J2A24), 10L (BDN: J2A7, J2A11, J2A12, J2A13, J2A14, J2A17, J2A18) as holotype; 2♂♂ (BDN & BOLD Sample ID: XS1, XS2), 1♀ (BDN & BOLD Sample ID: XS3), 1L (BDN & BOLD Sample ID: XS4), Zhejiang, Zhoushan, Xishan Island, seawater ponds, 29.896°N, 122.305°E, 1 m a.s.l., 5.VI.2013, hand net, leg. X. Qi; 1♂ (NTNU-VM & BOLD Sample ID: XL554), 1♀ (BDN & BOLD Sample ID: XL557), 3Pe (BDN: XL558, XL559, XL560), Zhejiang, Taizhou, Sanmen, Huaqiaozhen, marine ponds, 28.9375°N, 121.5221°E, 5 m a.s.l., 30.IV.2016, hand net, leg. B.J. Yang.

Etymology. Named after the country of the type locality, China, '*sinicus*'; adjective in nominative case.

Diagnosis. Morphological features of the new species do not fit with the generic diagnosis of *Dicrotendipes* and the remaining known genera of Chironomini. Adult male: antenna with ten flagellomeres, lacking plume; eye without microtrichia between ommatidia, without dorsomedial extension; palp with five segments, segment 4 and 5 occasionally slightly fused; brachypterous, shortened wings oar-shaped, with tuft of long setae apically; squama glabrous; acrostichals absent; mid and hind tibiae both with two spurs, and additional few small teeth; inferior volsella extremely long, strongly curved, extending beyond the apex of gonostylus; gonostylus moveable, with a few teeth distally. Adult female: antenna with six flagellomeres, without plume; palp with three segments; acrostichals usually absent, sometimes 1–5; mid tibia with two spurs and few small teeth, hind tibia with one spur and small teeth. Pupa: thoracic horn plumose, divided to numerous fine branches; basal ring larger, kidney-shaped, with two separated tracheal marks; pedes spurii B absent; segments V–VIII with 4 LT. Larva: antenna 5-segmented; mentum with rounded, trifid median tooth and six pairs

of lateral teeth, regularly decreasing in size laterally; premandible with four teeth; ventromental plates strongly curved; body without lateral or ventral tubules.

Description.

Adult male (n = 10, unless otherwise stated)

Total length 2.60–4.48, 3.16 mm. Wing length 1.09–1.68, 1.29 mm. Total length/wing length 1.61–3.44, 2.48.

Coloration (Figs S1–S2). Head, legs and abdomen brown. Basic color of thorax brown, with darkened patches posteriorly on scutum, scutellum and basal part on postnotum; median region of antepronotum dark brown.

Head (Fig. S2B). Eye glabrous, without dorsomedial extension. Frontal tubercles 5–15, 10 μm long, 5–20, 12 μm wide. Temporal setae 8–11, 9, including 0–3, 2 inner verticals, 1–10, 5, outer verticals and 1–3, 2 postorbitals. Clypeus with 16–26, 21 setae. Tentorium 93–135, 115 μm long, 13–25, 20 μm wide. Palp five-segmented, shortened; segment 3 without subapical sensilla; segment 4 and 5 incompletely fused in some specimens. Palpomere lengths (in μm): 25–50, 40; 40–60, 49; 80–105, 92; 25–60, 49; 30–70, 52.

Antenna (Figs S2A; S6A). Antenna with ten flagellomeres; ultimate flagellomere with several subapical sensilla (Fig. S2A). AR 0.96–1.43, 1.18. Plume absent, but surface of antenna covered with short setae and microtrichia.

Thorax (Figs S2C; S6B). Antepronotal lobes moderately developed, dorsally separated. Scutum not protruding beyond antepronotum, with tubercle. Acrostichals absent; dorsocentrals 9–14, 13; prealars 4–6, 5; scutellars 4–10, 7. Halter large (Fig. S2C).

Wing (Fig. S3A). Reduced in size, oar-shaped, bifurcated and with tuft of long setae apically; venation indistinct in some specimens. Membrane without setae, but with numerous microtrichia. Costa not produced beyond R_{4+5}; R_{2+3} ending close in distal 1/5 between apices of R_1 and R_{4+5}; R_{4+5} ending distal to apex of M_{1+2}; FCu far distal to RM, ending in proximal to apex of R_{4+5}. VR 1.46–1.83, 1.62. Brachiolum with one seta, R with 7–13, 10 setae; R_1 with 8–13, 11 setae; R_{4+5} with 0–1 seta; remaining veins glabrous. Anal lobe small. Squama glabrous.

Legs (Fig. S4A-C, G-I). Fore leg (Fig. S4A) very long relative to mid (Fig. S4B) and hind legs (Fig. S4C); apex of fore tibia (Fig. S4G) without spur; mid tibia (Fig. S4H) with two short, robust spurs and 3–7 teeth; hind tibia (Fig. S4I) with two short, robust spurs and 0–2 teeth, covered with three types of setae, short setae on inner margin, short but stout setae as spines on outer margin and long setae distally; all tarsomeres normally developed on fore leg but reduced on mid and hind legs. Claws normally developed, with a pointed spine. Pulvilli present. Lengths (in μm) and proportions of legs as in Table 1.

Table 1. Lengths (in μm) and proportions of legs of *Dicrotendipes sinicus* Qi & Lin **sp. n.**, male (n = 10)

	fe	ti	ta_1	ta_2	ta_3	ta_4
p_1	780–1050, 892	670–875, 750	1100–1425, 1225	690–900, 792	590–770, 680	490–640, 555
p_2	335–570, 489	370–600, 514	115–175, 135	48–63, 54	35–53, 44	30–45, 38
p_3	660–920, 753	540–740, 653	133–170, 151	40–65, 53	40–54, 49	35–53, 39

	ta_5	LR	BV	SV	BR
p_1	200–240, 219	1.57–1.80, 1.64	1.20–1.31, 1.28	1.24–1.47, 1.34	0.79–1.54, 1.02
p_2	35–75, 64	0.19–0.32, 0.27	4.55–6.61, 5.69	5.97–8.97, 7.47	0.53–0.85, 0.68
p_3	63–75, 68	0.20–0.26, 0.23	6.34–8.11, 7.48	8.35–10.22, 9.32	0.46–1.39, 0.73

Hypopygium (Figs S5C, D; S6C, D). Anal tergite bands strongly developed, fused anteriorly. Anal tergite with 46–62, 50 moderately long setae medially and posteriorly. Anal point robust and broadly rounded, 73–100, 84 μm long, with several moderately long setae. Superior volsella absent. Median volsella broadly digitiform, with 8–13, 10 long setose lamellae, covered with numerous microtrichia. Inferior volsella extremely long, 240–305, 262 μm long, strongly curved, extending beyond apex of gonostylus, distally rounded, with 21–52, 42 setae and numerous microtrichia. Gonocoxite 205–340, 252 μm long. Gonostylus short, 158–195, 172 μm long, with

File S1. Primers used for polymerase chain reaction amplification and sequencing.

Gene	Name	Sequence	Reference
18S rDNA	18S_ai	CCTGAGAAACGGCTACCACATC	Whiting et al. (1997)
18S rDNA	18S_bi	GAGTCTCGTTCGTTATCGGA	Whiting et al. (1997)
28S rDNA	S3660	GAGAGTTMAASAGTACGTGAAAC	Morse & Normark (2006)
28S rDNA	A335	TCGGAAGGAACCAGCTACTA	Whiting et al. (1997)
CAD1	54F	GTNGTNTTYCARACNGGNATGGT	Moulton & Wiegmann (2004)
CAD1	405R	GCNGTRTGYTCNGGRTGRAAYTG	Moulton & Wiegmann (2004)
CAD4	787F	GGDGTNACNACNGCNTGYTTYGARCC	Moulton & Wiegmann (2004)
CAD4	1098R	TTNGGNAGYTGNCCNCCCAT	Moulton & Wiegmann (2004)
COI mtDNA	LCO1490	GGTCAACAAATCATAAAGATATTGG	Folmer et al. (1994)
COI mtDNA	HCO2198	TAAACTTCAGGGTGACCAAAAAATCA	Folmer et al. (1994)
COI mtDNA	s2183	CAACATTTATTTTGATTTTTTGG	Simon et al. (1994)
COI mtDNA	a3014	TCCAATGCACTAATCTGCCATATTA	Simon et al. (1994)

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File S3. List of taxa, codes, life stages, locations and GenBank accessions

Species	ID	Life stage	Locations	28S	COI-3P	18S	CAD1	CAD4
Axarus sp. 1	AXAR1	L	USA, Massachusetts, Connecticut R. (S. Werle)	HQ440713	HQ440877	HQ440565	HQ440246	HQ440422
Chironomini sp. 1	TH051	L	Thailand, Phang Nga, Sri Phang Nga NP	HQ440854	HQ441016	HQ440693	HQ440392	HQ440544
Chironomus sp. 1	ChV1	L	USA, California, Solano, nr Vacaville	HQ440719	HQ440883	HQ440572	HQ440256	HQ440428
Cryptochironomus sp. 1	MODOC6	L	USA, California, Ash Valley lake exit	HQ440729	HQ440893	HQ440582	HQ440266	HQ440438
Cryptotendipes sp. 1	MODOC5	L	USA, California, Ash Valley mud lake	HQ440730	HQ440894	HQ440583	HQ440267	HQ440439
Dicrotendipes peringueyanus	Dper	L	South Africa, Eastern Cape, Gt Fish R.	HQ440732	HQ440896	HQ440585	HQ440270	HQ440440
Dicrotendipes sp. 1	N107	L	Australia, New South Wales,Captain's Flat, Molonglo R.	HQ440733	HQ440897	HQ440586	HQ440271	HQ440441
Dicrotendipes sp. 3	DIC3	L	USA, California, McClaughlin Reserve	HQ440734	HQ440898	HQ440587	HQ440272	HQ440442
Endochironomus sp. 1	NCA4	L	USA, Oregon, U. Klamath L.	HQ440739	HQ440903	HQ440591	HQ440277	HQ440446
Endochironomus sp. 2	MODOC4	L	USA, California, Madeline	HQ440740	HQ440904	HQ440592	HQ440278	HQ440447
Endotribelos sp. 1	CAM03	L	USA, California, Maillard Redwoods State Forest	HQ440741	HQ440905	HQ440593	HQ440279	HQ440448
Glyptotendipes sp. 1	NCA6	L	USA, California, Murphys Well	HQ440752	HQ440914	HQ440601	HQ440286	HQ440454
Harnischia sp. 1	HARN	L	Australia, Australian Capital Territory, Brindabellas	HQ440753	HQ440915	HQ440602	HQ440287	HQ440455
Harrisius sp. 1	V604	L	Australia, Victoria, Towonga, Mt Bogong, Mountain Ck.	HQ440754	HQ440916	HQ440603	HQ440288	HQ440456
Harrisius sp. 2	NZ07.4.5	L	New Zealand, South I.,, Tasman-Nelson, Hope R.,	HQ440755	HQ440917	HQ440604	HQ440289	HQ440457
Imparipecten pictipes	N103	L	Australia, New South Wales,Captain's Flat, Molonglo R	HQ440759	HQ440921	HQ440608	HQ440294	HQ440461
Imparipecten sp. 2	TH054	L	Thailand, Prachuap Khiri Khan, Kui Buri	HQ440760	HQ440922	HQ440609	HQ440295	HQ440462
Kiefferulus barbatitarsis	TH03	L	Thailand, Ranong, Leam Son NP	HQ440762	HQ440923	HQ440610	HQ440297	N/C
Kiefferulus calligaster	KIEF2P	Pf	Thailand, Post-tsunami ponds	HQ440763	HQ440924	HQ440611	HQ440298	HQ440464
Nilothauma sp. 1	AUNT02	L	Australia, Northern Territory, Magela Ck. (Humphrey)	HQ440782	HQ440945	HQ440629	HQ440316	HQ440481
Pagastiella sp. 1	TH35	L	Thailand, Phu Hin Rhomkia NP, Waterwheel	HQ440788	HQ440951	N/C	HQ440321	N/C
Parachironomus sp. 1	NCA2	L	USA, Oregon, U. Klamath L.	HQ440791	HQ440954	HQ440635	HQ440323	HQ440486
Parachironomus sp. 2	MODOC3	L	USA, California, Madeline	HQ440792	HQ440955	HQ440636	HQ440324	HQ440487
Paracladopelma sp.	FNQ9.1	L	Australia, Queensland, Mary Ck.	HQ440793	HQ440956	HQ440637	HQ440325	HQ440488
Polypedilum vanderplanki	PVP3	L	W. Africa, ex- Culture (Japan, ex. W. Africa)	HQ440816	HQ440976	HQ440656	HQ440350	HQ440509

Polypedilum sp. 1	SAPP1	L	South Africa, W. Cape, Burgerskloof	HQ440813	HQ440977	HQ440657	HQ440346	HQ440506
Polypedilum sp. 2	FNQ4.2	Pm	Australia, Queensland, Nigger Ck.	HQ440815	HQ440979	HQ440659	HQ440349	HQ440508
Polypedilum sp. 3	FNQ7.5	L	Australia, Queensland, Davies Ck.,	HQ440811	HQ440974	HQ440654	HQ440344	HQ440504
Polypedilum prasiogaster	V403	L	Australia, Victoria, Buckland S.F., U. Buckland R.,	GU356717	GU356707	GU356735	HQ440348	GU356689
Shangomyia impectinata	SHANGO	L	Thailand, Loie, Na Haew NP, International Falls	HQ440830	HQ440993	HQ440671	HQ440366	HQ440524
Stenochironomus sp. 3	STENO	L	Australia, Queensland, Oliver Ck., Daintree	HQ440837	HQ441000	HQ440677	HQ440372	HQ440529
Stenochironomus sp. 4	STMA1	L	USA, Massachusetts, Ashuelot R. near Hinsdale, (S. Werle)	HQ440838	HQ441001	HQ440678	HQ440373	HQ440530
Stictochironomus sp. 1	FNQ7.11	L	Australia, Queensland, Davies Ck.	HQ440840	HQ441003	HQ440680	HQ440375	HQ440532
Stictochironomus sp. 2	TH56	L	Thailand, Petchaburi, Nhum Sao Falls	HQ440841	HQ441004	HQ440681	HQ440376	HQ440533
Xestochironomus sp. n.	CH 13.1	m	Chile, IX region, Peulla, behind hotel	HQ440861	HQ441023	HQ440700	HQ440401	HQ440549
Xylochironomus kakadu	AUNT04	L	Ausralia, Northern Territory, Magela Ck.	HQ440863	HQ441025	HQ440702	HQ440403	HQ440551
Xiaomyia sp. 1	XIAO	m	Thailand, Phang Nga, Sri Phang Nga NP.	HQ440862	HQ441024	HQ440701	HQ440402	HQ440550
Axarus fungorum	CH366	m	China, Guangxi, Luocheng, Xiaochang'anzhen	MK074888	MK074901	MK074978	MK074914	MK074938
Dicrotendipes flexus	CHCH175	m	China, Liaoning, Dandong, Kuandian, Qianshan Lake	MK074890	MK074903	MK074980	MK074917	MK074940
Dicrotendipes nervosus	CH216	m	China, Heilongjiang, Jixi, Xingkai Lake	MK074887	MK074900	MK074977	MK074913	MK074937
Dicrotendipes nudus	CH275	m	China, Hainan, Wuzhishan, Shuimanxiang, Tropical Froest Park	MK074885	MK074898	MK074975	MK074911	MK074935
Dicrotendipes pelochloris	CH213	m	China, Jilin, Yanbian, Fusong, Nenjiang park	MK074892	MK074905	MK074982	MK074920	MK074941
Dicrotendipes saetanumerosus	CH364	m	China, Zhejiang, Quzhou, Qujiang, Wuxijiang	MK074893	MK074906	MK074983	MK074921	MK074942
Dicrotendipes septemmaculatus	CH348	m	China, Shandong, Jining, Weishan, Weishan island	MK074886	MK074899	MK074976	MK074912	MK074936
Dicrotendipes sp. 2XL	CH186	m	China, Heilongjiang, Jixi, Xingkai Lake	MK074896	MK074909	MK074986	MK074924	MK074945
Dicrotendipes sp. 3XL	CH363	m	China, Zhejiang, Wenzhou, Yueqing	MK074891	MK074904	MK074981	MK074918	N/C
Dicrotendipes tritomus	CH219	m	China, Inner Mongolia, Toudaoqiao	MK074895	MK074908	MK074985	MK074923	MK074944
Dicrotendipes sinicus sp. n.	XS1	m	China, Zhejiang, Zhoushan, Xishan Island	MK074897	MK074910	MK074987	MK074925	MK074946
Fleuria lacustris	CH150	m	China, Inner Mongolia, Hulunbeier, Honghuaerjizhen	MF828494	MF828493	MF828495	MK074915	MF828492
Kiefferulus tainanus	CH159	m	China, Henan, Xinxiang, Muye district, Wei River	MK074889	MK074902	MK074979	MK074916	MK074939

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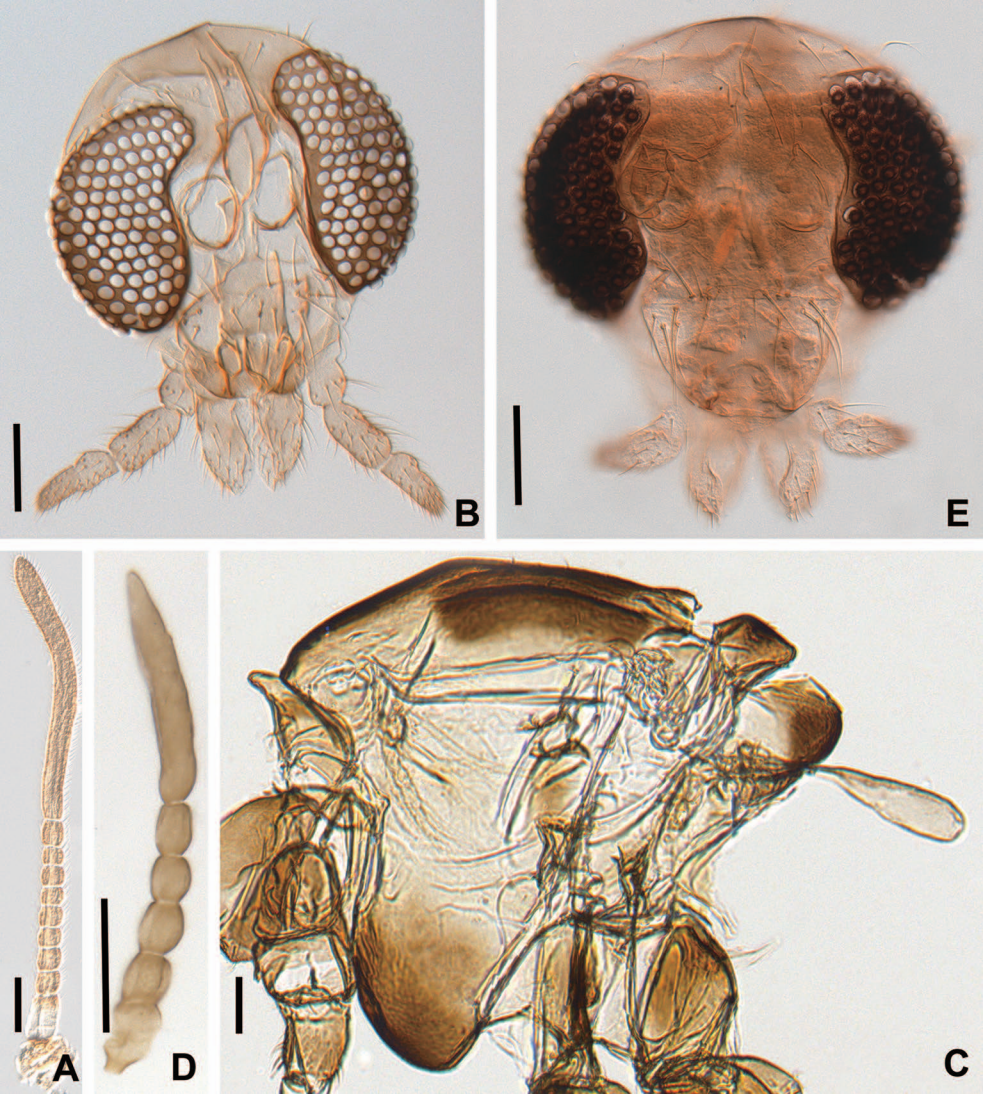
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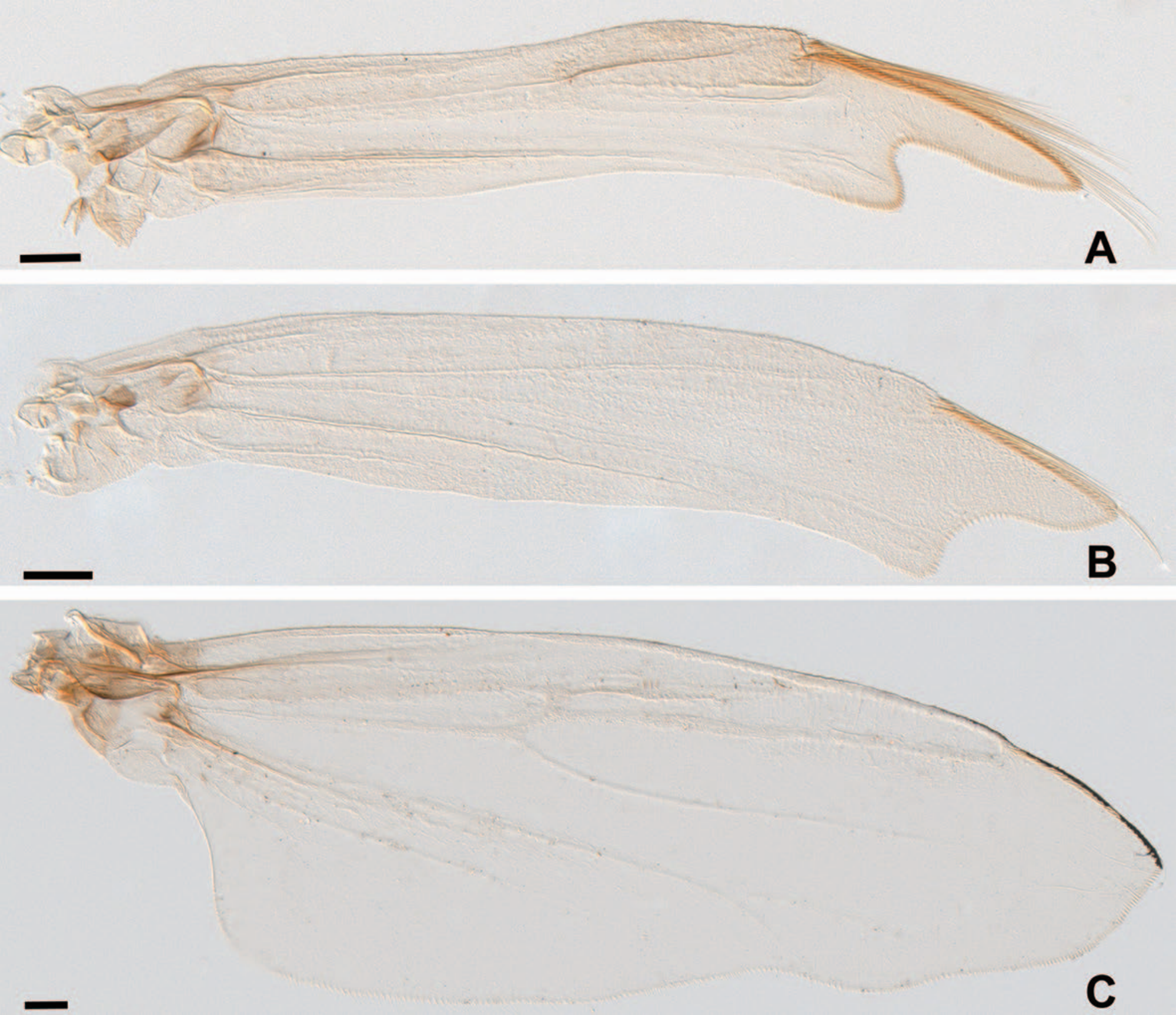
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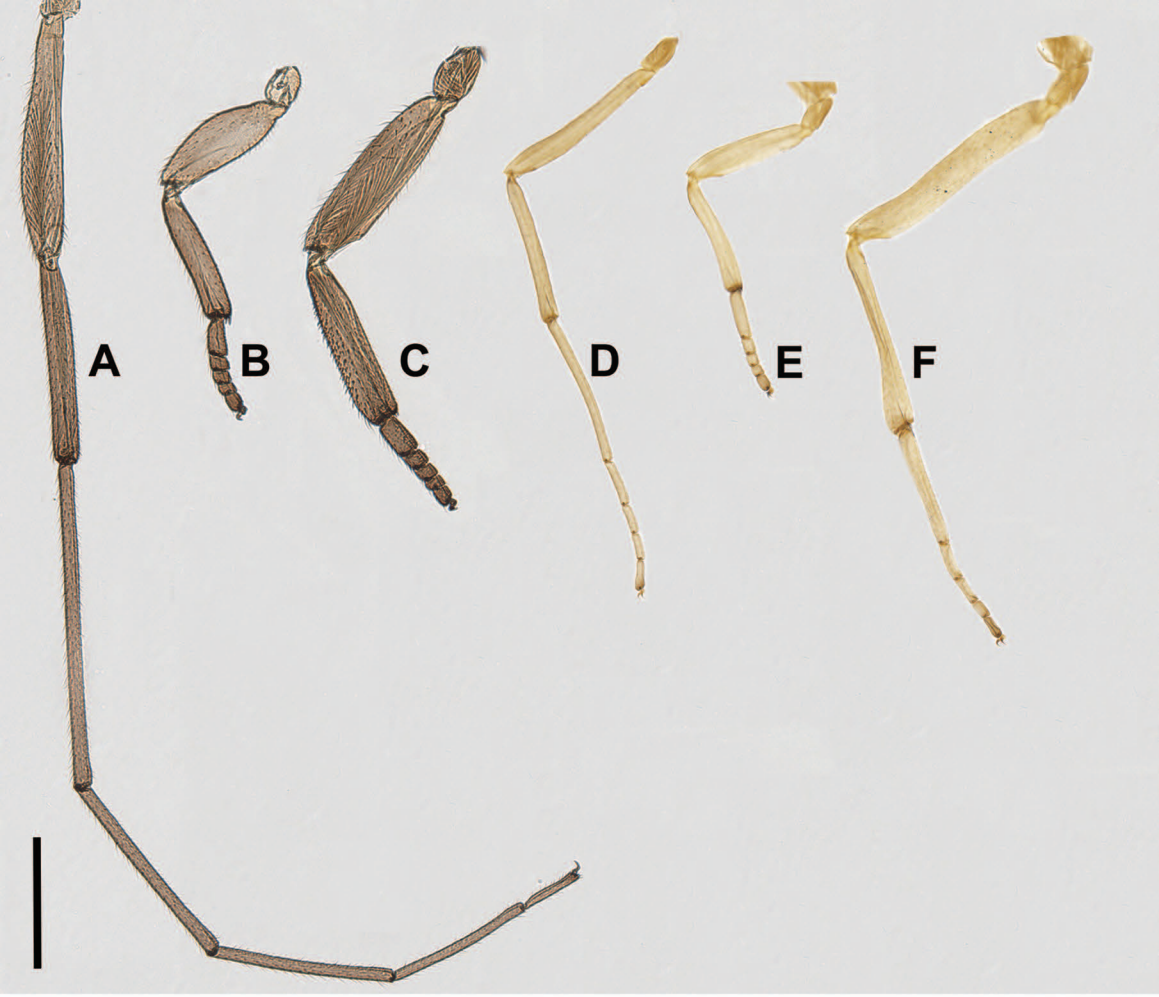
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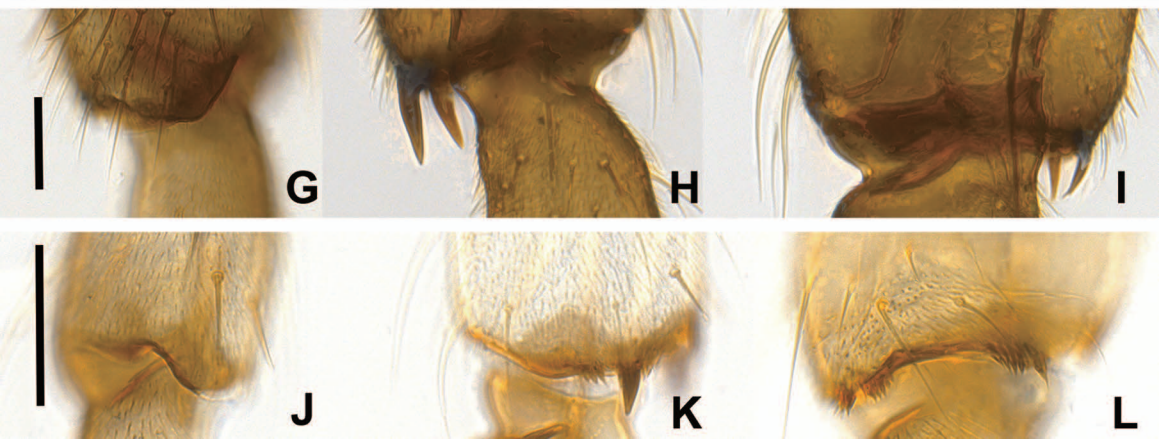
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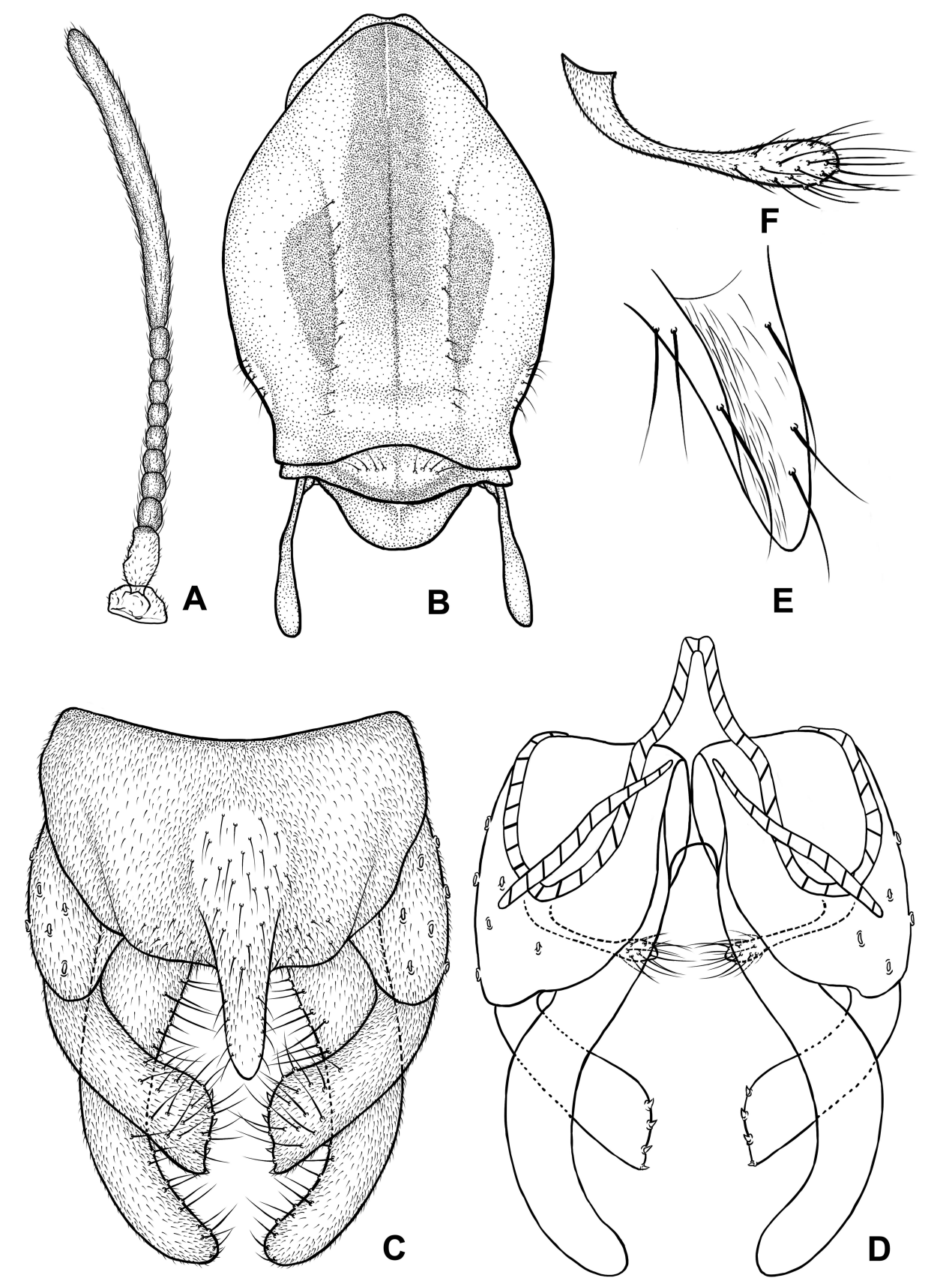
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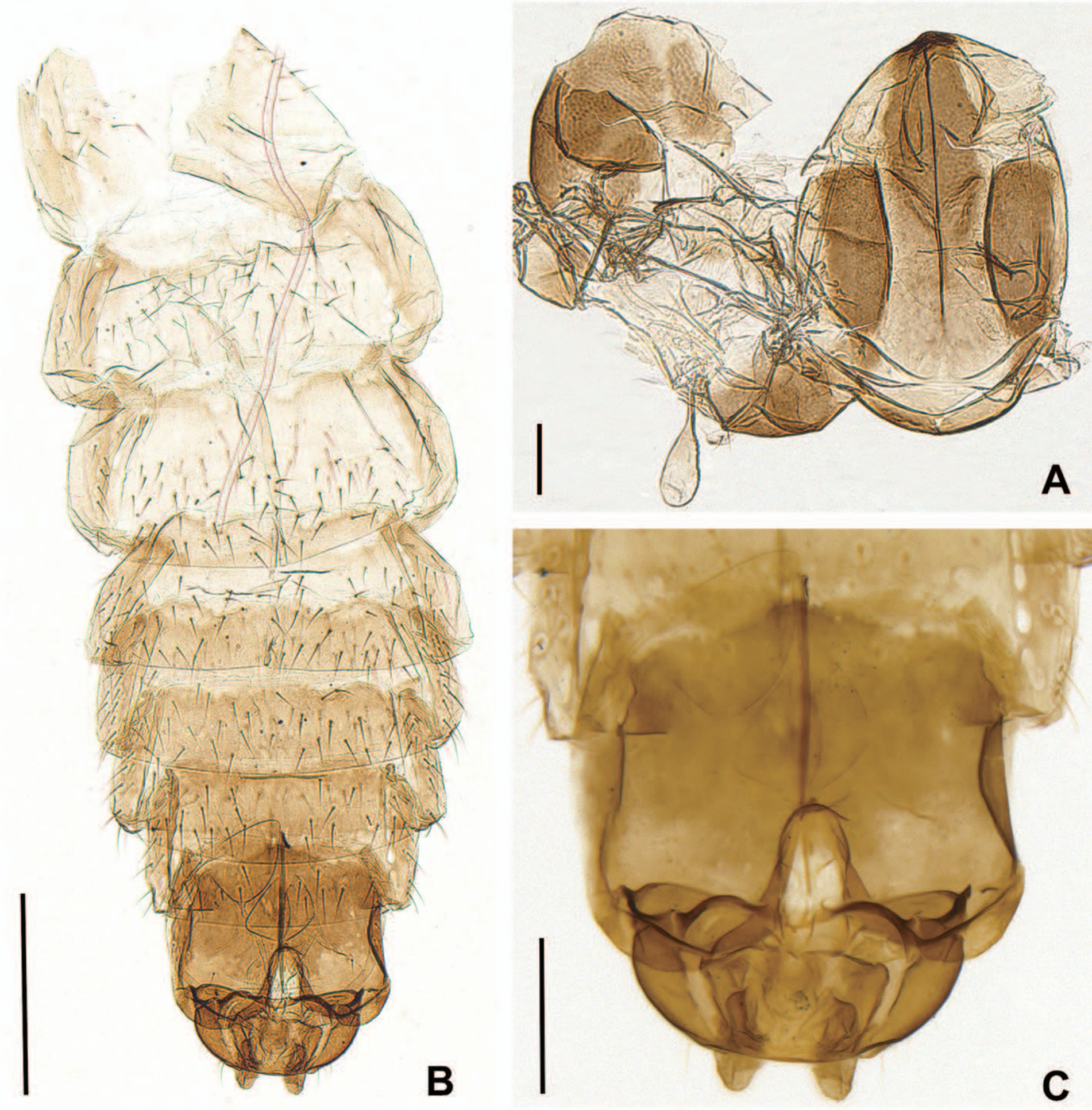
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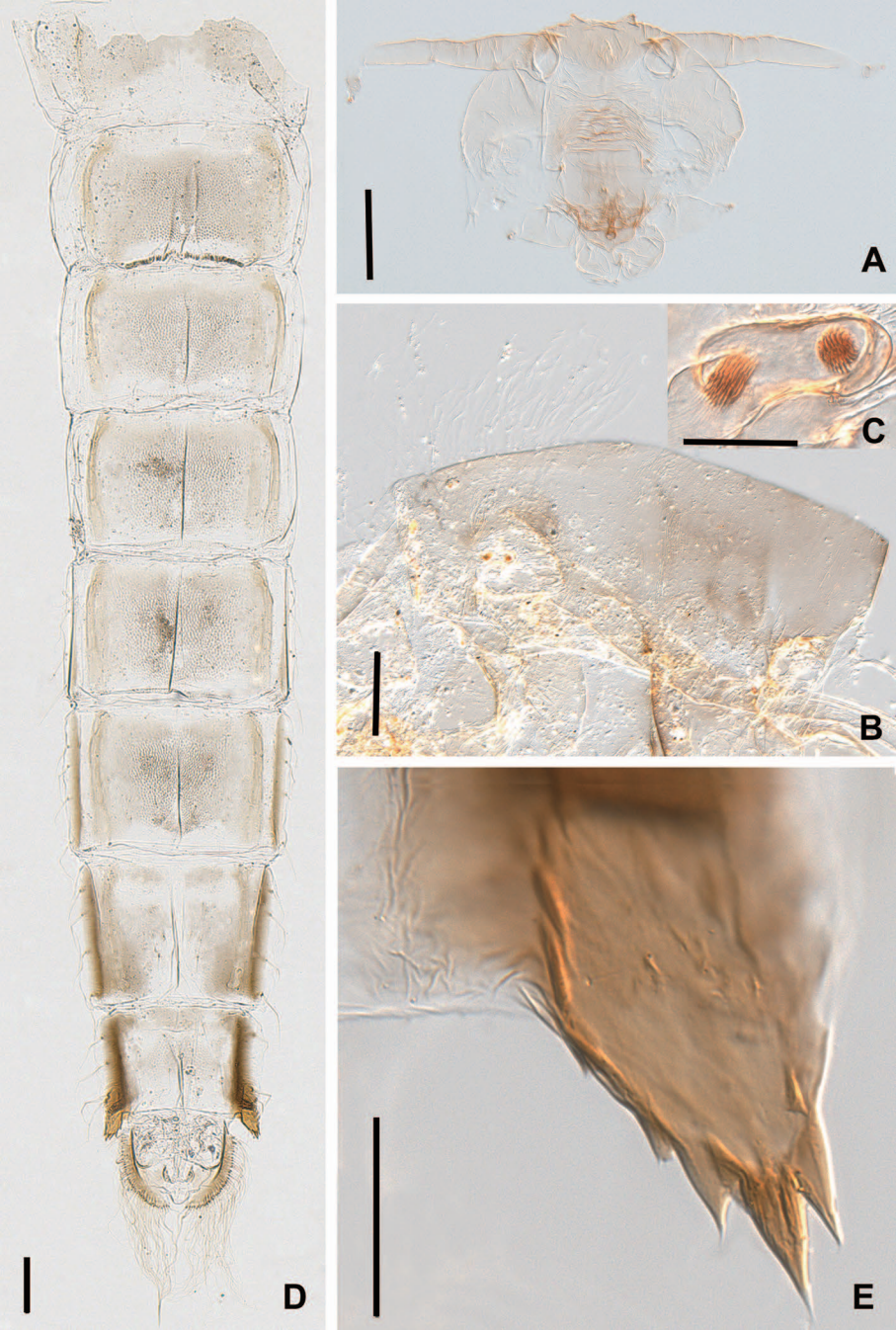
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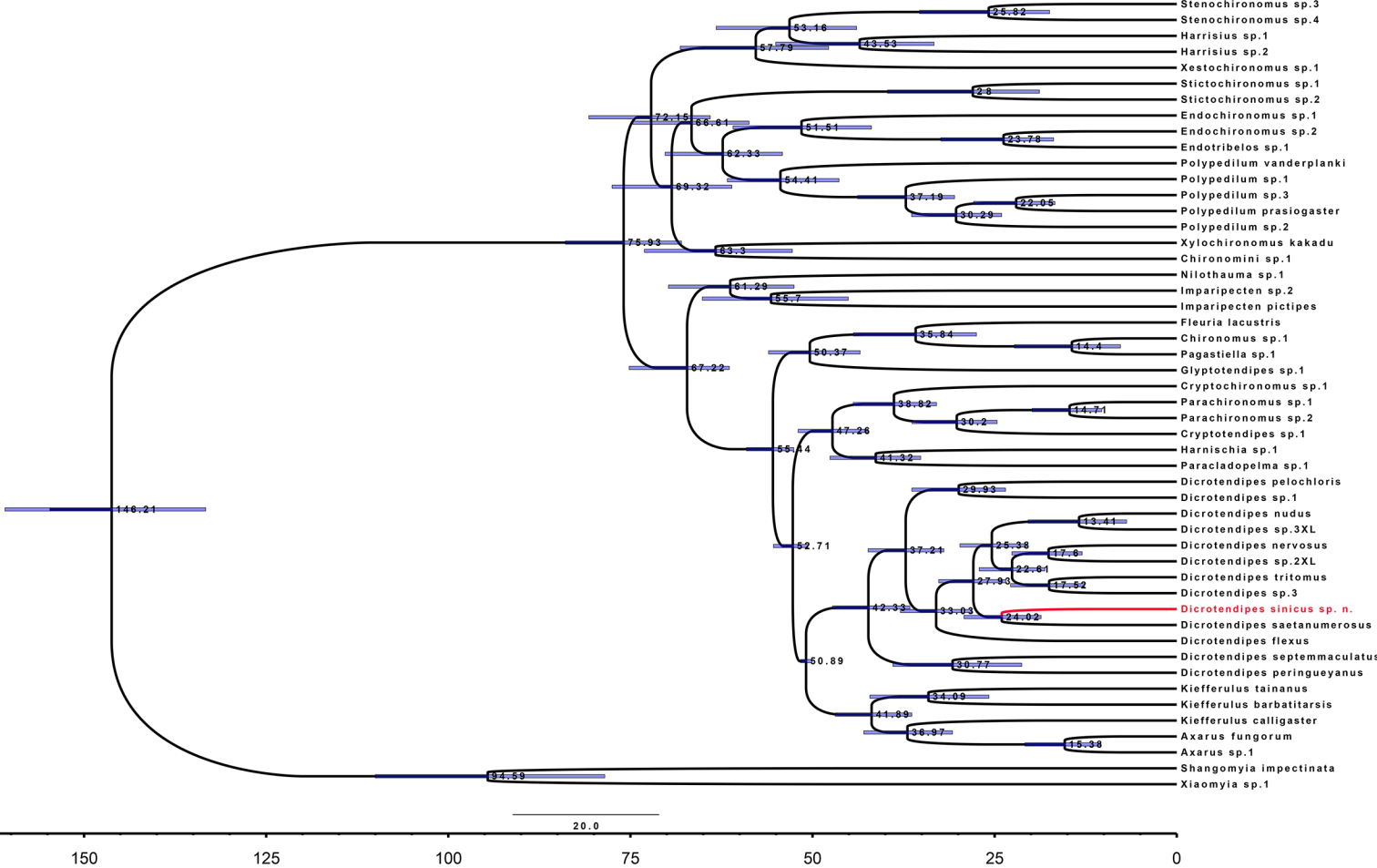
Stenochironomus sp.3
Stenochironomus sp.4
Harrisius sp.1
Harrisius sp.2
Xestochironomus sp.1
Stictochironomus sp.1
Stictochironomus sp.2
Endochironomus sp.1
Endochironomus sp.2
Endotribelos sp.1
Polypedilum vanderplanki
Polypedilum sp.1
Polypedilum sp.3
Polypedilum prasiogaster
Polypedilum sp.2
Xylochironomus kakadu
Chironomini sp.1
Nilothauma sp.1
Imparipecten sp.2
Imparipecten pictipes
Fleuria lacustris
Chironomus sp.1
Pagastiella sp.1
Glyptotendipes sp.1
Cryptochironomus sp.1
Parachironomus sp.1
Parachironomus sp.2
Cryptotendipes sp.1
Harnischia sp.1
Paracladopelma sp.1
Dicrotendipes pelochloris
Dicrotendipes sp.1
Dicrotendipes nudus
Dicrotendipes sp.3XL
Dicrotendipes nervosus
Dicrotendipes sp.2XL
Dicrotendipes tritomus
Dicrotendipes sp.3
Dicrotendipes sinicus sp. n.
Dicrotendipes saetanumerosus
Dicrotendipes flexus
Dicrotendipes septemmaculatus
Dicrotendipes peringueyanus
Kiefferulus tainanus
Kiefferulus barbatitarsis
Kiefferulus calligaster
Axarus fungorum
Axarus sp.1
Shangomyia impectinata
Xiaomyia sp.1
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