

Description of larva of *Anax ephippiger* (Burmeister, 1839) from Japan, including changes and developments in external morphology (Odonata: Aeshnidae)

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Abstract: The external morphology of larva of *Anax ephippiger* (Burmeister, 1839) from Japan is described as follows: change of morphology and coloration/markings from 1st to final instar; the detail external morphology of final instar; developmental change of final instar larva. These are compared with the previous studies of this species, as well as other species of *Anax*.

Key words: Anisoptera, dragonfly, Anactini, egg, instar, comparison.

Introduction

Anax ephippiger (Burmeister, 1839) primarily inhabits in arid areas of Africa, southern Europe, the Middle East and Pakistan (Askew, 2004), but often actively migrates to surrounding regions and temporarily breeds the destinations (Kosterin & Borisov, 2018). In Japan, this species was first collected in Hamamatsu City, Shizuoka Pref. in 1996 (Ugai, 1996), and only 7 males and 3 females were recorded until 2017 (Ugai & Yamashita, 2019). In autumn 2019, 4 males and 4 females were recorded at several locations from Toyama Prefecture to Yakushima Island (Sasamoto & Futahashi, 2021; Suzuki *et al.*, 2021; Saito & Futahashi, 2022).

The external morphology of larva of this species has been illustrated based on individuals from Israel (De Marmels, 1975), Ukraine, and Nigeria (Butler, 1998) as a detailed description. In addition, the larva of the allied species, *Anax papuensis* (Burmeister, 1839), which was once treated under the genus *Hemianax* together with *ephippiger*, was described by Rowe (1991) based on materials from New Zealand. However, the larva of *A. ephippiger* has not been described from Asia. In this study, we observed morphology of larvae reared from eggs that were obtained from an adult female collected in Enshû-hama, Hamamatsu City, Shizuoka Pref., 5-XI-2019 by Kotabe (Kotabe *et al.*, 2020). Here we describe the external morphology of young instar to final instar, and change of compound eyes of final instar of *A. ephippiger*.

The terminology mainly follows Kawashima & Sasamoto (2007). The abbreviations used in this paper are as follows: A– average; AK– Akira Kotabe; AS– Akihiko Sasamoto; AW– maximum width of abdomen, on 7th segment; BL– body length, from anterior margin of labrum to the apex of paraprocts; F– final larval instar; F-1 (= F minus 1)– penultimate larval instar; GO– Genta Okude, HW– head capsule width, across compound eyes; HWL– length of hindwing sheath, on the upper margin; HTL– length of femur of metalegs, on the upper margin; PW– maximum width of pronotum; RF– Ryo Futahashi, S– abdominal segment; YT– Yuichi Takuma.

Change of morphology and coloration/markings (Yuichi TAKUMA)

Materials and method

One hundred thirty-seven eggs were offered for YT to rear, which was the part of the eggs laid from an adult female captured by AK (Kotabe *et al.*, 2020). Among them, 126 eggs succeeded to hatch and continued to develop, though decreasing in number; eventually, six larvae survived to emerge. Here, we treat the larva next to prolarva as instar 1, as most Japanese publications (e.g., Watanabe, 1996), although Corbet (1999) insisted on the rationality of treating prolarva as instar 1.

In principle, the earliest molted larva from each instar stage was selected and photographed. The photos were made using a digital camera with macro lenses, but Fig. 1B was done by a compact digital camera equipped with a “microscopic mode” and focus stacking technique. Because the images are not precise enough to figure out the microstructures, we mainly describe the developmental changes based on rough morphology and coloration/markings.

Description.

In this paper, instars of similar coloration are categorized and described because the color pattern changes markedly during development, as was also mentioned in Calvert (1934), Corbet (1955), and Rowe (1991). In this series, the earliest molted larva was recognized to grow to the 11th as final instar. However, the number of larval instars is not fixed like many Odonata species (Corbet, 1999) and many other larvae needed one or two more molting to reach the final instar.

General external morphology is similar throughout all instar stages as most larvae in Aeshnidae (Corbet, 1999); large head with well-developed compound eyes; thorax of 3 segments narrower than head; elongate abdomen being slender at the basal segments gradually widening to S7, then tapering to anal appendages. The overall coloration change is summarized as from the early “dark and light banded pattern” to the late “greenish ground color with pale marbling pattern.”

Instar 1 (Fig. 1A, D, F, I): Coloration and markings pattern appear to be homogeneous among individuals. Its general coloring is dark brown with several pale yellowish markings. Head bears a central cross-shaped marking that continues to a downward-arrow-shaped marking on body, ending at distal margin of abdominal S3. The following morphology on head is noteworthy. The dome-shaped expansion on anterior margin (Fig. 1F, a black arrowhead) is unique to

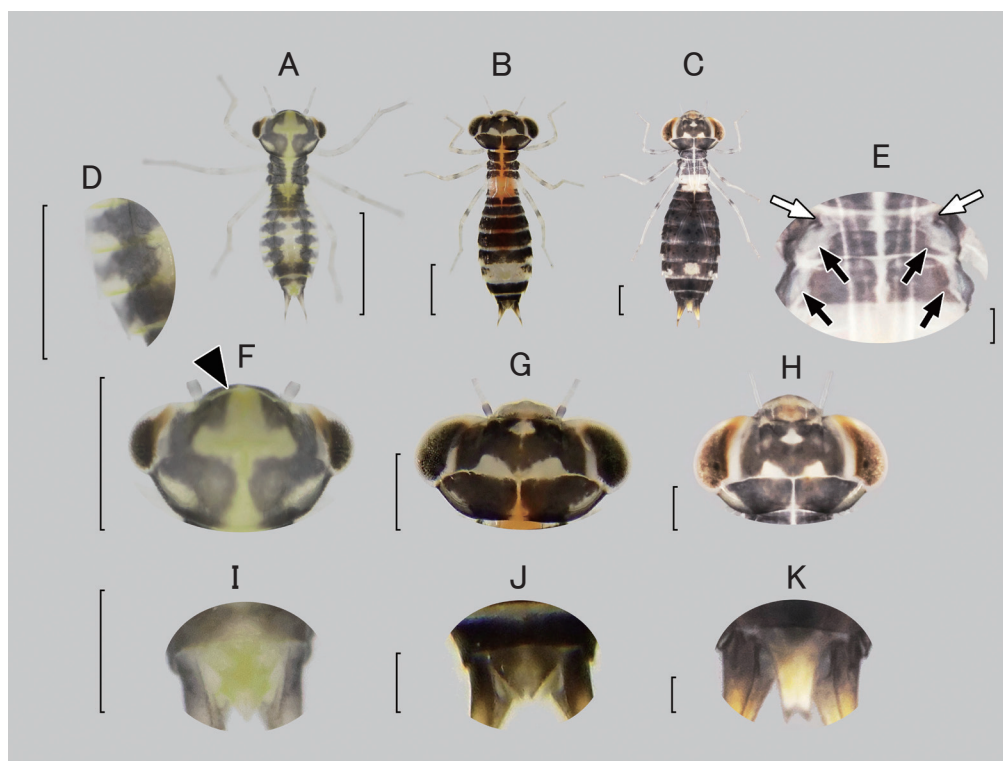


Fig. 1. Larvae of *Anax ephippiger* in dorsal view. A–C. General appearance in instar 1, 3, and 5, respectively. The orange color on the body in instar 3 is fed on *Artemia*; D. Left lateral margin of abdominal S7-9 in instar 1; E. Thorax in instar 5. Black arrows indicate wing sheaths. White arrows indicate mesopleura; F–H. Head in instar 1, 3, and 5, respectively. Black arrowhead indicates the dome-shaped expansion in instar 1; I–K. Epiproct in instar 1, 3, and 5, respectively. Scales: 1.0 mm (A–C); 0.5 mm (D, F–H); 0.2 mm (E, I–K).

instar 1, which probably facilitates rupture of the chorion while hatching (Corbet, 1999). In addition, compared with the succeeding stages, occiput is more extensive, compound eyes smaller, and their inner margins are nearly parallel. Femora have a vague narrow annular marking distally, and tibiae have a similar one proximally. Abdomen has a broad central stripe of varying width on S6-8 and a lateral jagged marking on each segment. Lateral spines are present on S8, 9, and probably on S7 (Fig. 1D), all of which are short and small. Epiproct is triangularly likely with a cleft on its apex. Paraprocts are elongate, sharply pointed and weakly incurved apically. Cerci are hard to identify in this instar.

Instar 2–5: These stages (Fig. 1B, C, E, G, H, J, K) show a remarkable change in coloration from instar 1, i.e., the noticeable contrast between dark brown body and two broad pale bands on proximal abdomen and S8, so-called “banded” color pattern (Rowe, 1991). As for S8, shape of the band varies from oval to transverse band among individuals. In addition, there are pale, thin lines along with intersegmental articulations. The cross-shaped marking on head is separated into three small pale spots along two thin lines on the occipital sutures.

Compound eyes extend laterally, inner margins of which diverge posteriad. Frons protrudes further anteriorly. Wing sheaths are first seen as triangularly in instar 5 (Fig. 1E, black arrows). Small mesopleura begin to appear, widely separated from each other dorsally (Fig. 1E, white arrows). Femora and tibiae have similar markings as those of instar 1. Lateral spines of abdomen are present on S8, 9, and definitely on S7. Before instar 4, the shape of epiproct is almost same as that of instar 1, but its apex becomes elongate and bifid after instar 5. Paraprocts are similar with those of instar 1. Cerci are so tiny to identify.

Instar 6 (Fig. 2A, D): Coloration and markings are intermediate between instar 5 (Fig. 1C) and 7 (Fig. 2B). Its general coloring is dark brown, and traces of banding pattern are almost fading, except for an oval marking on S8. Epiproct

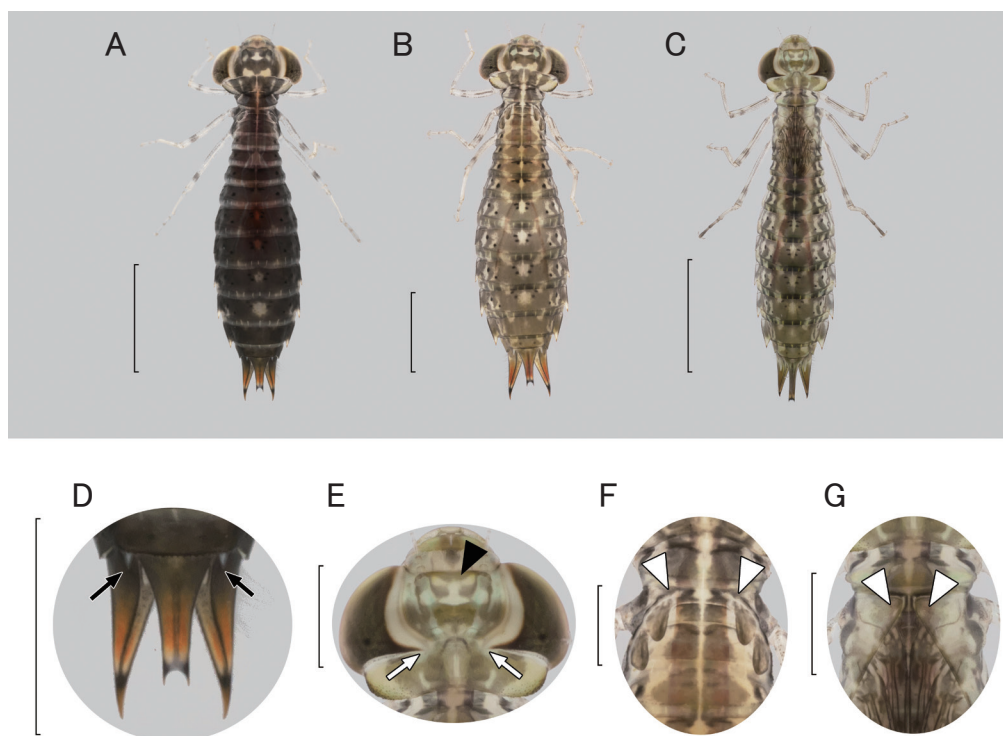


Fig. 2. Larvae of *Anax ephippiger* in dorsal view. A–C. General appearance in instar 6, 7, and 10 (= F-1), respectively; D. Anal appendages in instar 6. Black arrows indicate cerci; E. Head in instar 10. Black arrowhead indicates the markings on frons. White arrows indicate the inner-posterior points of compound eyes; F, G. Thorax in instar 7 and 10 (= F-1), respectively. White arrowheads indicate mesopleura. Scales: 3.0 mm (A, B, E & G); 10.0 mm (C); 1.0 mm (D, F).

elongates further, but still shorter than paraprocts. Finally, cerci are small, but visible (Fig. 2D, black arrows).

Instar 7–11 (Fig. 2B, C, E–G): Again, coloration pattern (Fig. 2B, C) remarkably changes to greenish/brownish ground color with many pale markings. Internal borders of compound eyes and lateral margins of occiput are pale; in addition, there are several small markings between these pale areas, especially a unique one on frons (Fig. 2E, a black arrowhead). Thorax has a thin longitudinal middle line, and two broad sinuous stripes in side. Middle of each abdominal segment bears a marking as follows; a thin longitudinal line (S1), a spindle shape with thin longitudinal line (S2–4), a spindle shape (S5, 6), a nearly round shape (S7, 8), a very faint round shape (S9). Laterally several symmetrical layers of various markings and stripes are found.

Some morphological features appear in this stage: i.e., the shape of compound eyes is characteristic as genus *Anax* (Butler, 1998); their lateral margins are elongated, twice of lateral margin of occiput. Inner-posterior point of each eye is gradually closer mesad (Fig. 2E, white arrows). Wing sheaths develop rapidly after instar 7, cover S1 at instar 9 (= F-2), then S1 and 2 at instar 10 (= F-1), finally reach the middle of S5 at instar 11 (= F). In earlier instars (Fig. 1E, white arrows & Fig. 2F, white arrowheads), right and left mesopleura are dorsally separated from each other, then, gradually extend further dorsad with each inner-anterior point in the lead, and finally almost touch along the mid-dorsal line in instar 10 (= F-1) (Fig. 2G, white arrowheads). Femora have the two annular markings. Epiproct elongates further, almost as long as paraprocts after instar 9, whose distal margin is concave. Cerci are sharply pointed, gradually elongated, finally about half of paraprocts in final instar.

External morphology of the final instar larva (Itsuro KAWASHIMA)

Specimens examined. 1♂4♀ exuviae (dried, some are pinned) of final instar larva (emergence data: 1♀, 24-V-2020; 1♂1♀, 2 to 3-VI-2020; 1♀, 7-VI-2020; 1♀, unknown). All were reared by AS and deposited in the same collection, except for the last one, which was reared by M. Fukui, through A. Ozono, and preserved in the IK collection.

Method.

All external characters were observed under a stereoscopic microscope (Olympus SZ 60, max. magnification $\times 160$ & SZH10, max. magnification $\times 140$) and were sketched with the aid of an equipped drawing tube attached to the later equipment by IK. For detailed examination, anterior part of the labium was removed off from the head capsule.

Measurements in mm. Male (n = 1) : BL: 39.10; HW: 7.60; PW: 4.60; HWL: 9.00; AW: 7.60; HTL: 6.25; Female (n = 4): BL: 38.0–43.10 (A 39.90); HW: 7.20–8.00 (A 7.58); PW: 4.40–4.80 (A 4.63); HWL: 8.20–9.00 (A 8.60); AW: 7.90–8.25 (A 8.01); HTL: 6.20–6.80 (A 6.53).

Description.

Coloration and marking (Fig. 3A, B). Body weakly shiny; color shading variable individually from dusty to yellowish brown. Body paler to yellowish brown, with markings on legs and abdomen. Each femur with two vague and paler annular markings on distal part. Abdominal tergites with two continuous dark longitudinal bands along center line, and geometrical markings especially on lateral part of each segment.

External morphology. General shape elongate as in most of the genus *Anax*. Body surface rather smooth and weakly shiny, with densely minute setae but seen like glabrous.

Head capsule (Fig. 4B) rounded and expanded laterally at compound eyes, and weakly and broadly depressed on dorsum. The length of head capsule slightly shorter than thorax, and the maximum width clearly wider than pterothorax. Antennae (Fig. 4A) seven-segmented, very short and filiform, shorter than half width of head capsule; relative length of each antennomere from scape (1st antennomere) as follows: 1.00: 1.23: 2.40: 0.93: 1.27: 1.27: 1.27; scape short and thick, feebly divergent toward the apex, as wide as long; pedicel a little longer than scape, barrel-shaped; each of basal 4 flagellar antennomeres (3rd to 6th) longitudinally cylindrical; 5th flagellar (terminal 7th) elongate spindle-shaped with a sharply pointed apex. Compound eyes large, rounded in dorsal view, expanded laterad, clearly longer than remaining side length of postocular lobes. Postocular lobes almost glabrous on dorsal surface; both sides feebly and obliquely convergent posteriad; both hind angles evenly rounded, lacking any processes; dorso-posterior areas vague longitudinally striae consisting of glabrous areas. Labrum short and narrow, 0.3 times as wide as maximum head width; lateral margins straight, clearly diverging anteriad, constricted basally; anterior margin arcuate, not concaved centrally. Anteclypeus short, almost as long as labrum; the surface rather smooth; anterior margin very shallowly produced anteriorly at center part; lateral margins abruptly convergent anteriad. Labium (Fig. 5) rather wide

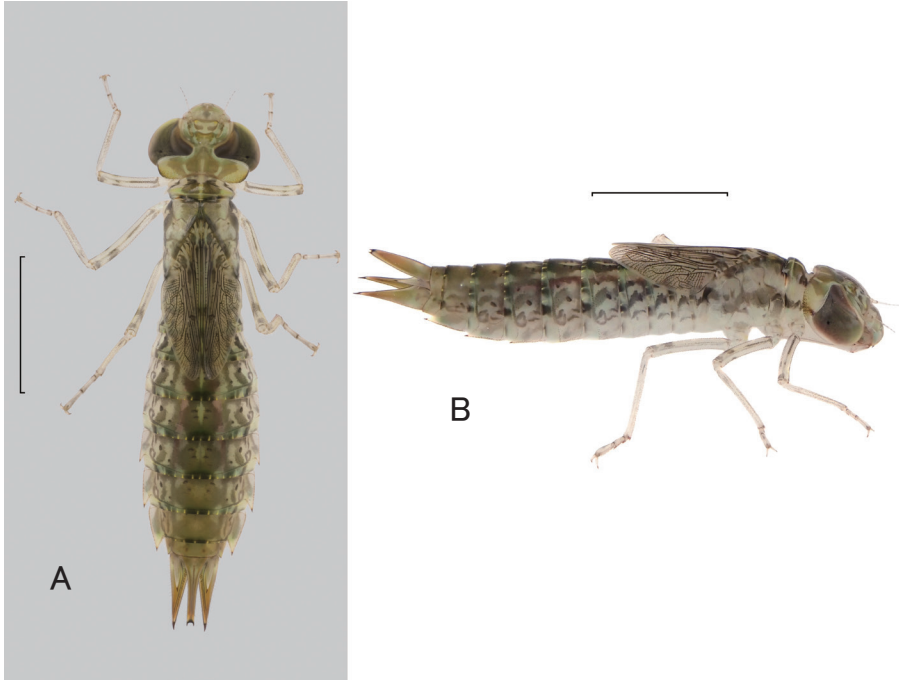


Fig. 3. Final instar larva of *Anax ephippiger*. A. General appearance in dorsal view; B. General appearance in lateral view. Scale: 1.0 cm.

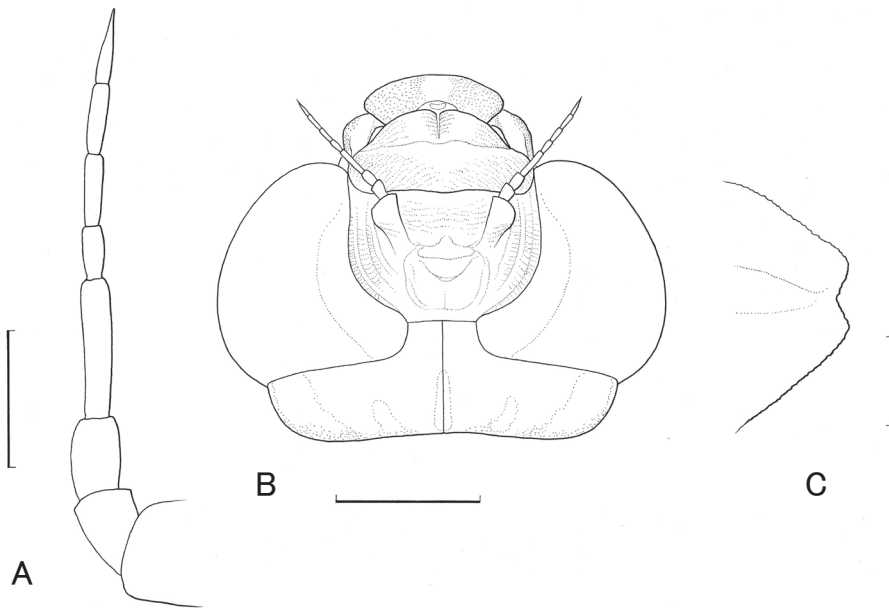


Fig. 4. Larval morphology of *Anax ephippiger*. A. Right antenna; B. Head capsule; C. Two processes on prothoracic pleura. All dorsal views. Scales: 1.0 mm (A); 2.5 mm (B); 0.5 mm (C). Del. KAWASHIMA Itsuro 2021 ©

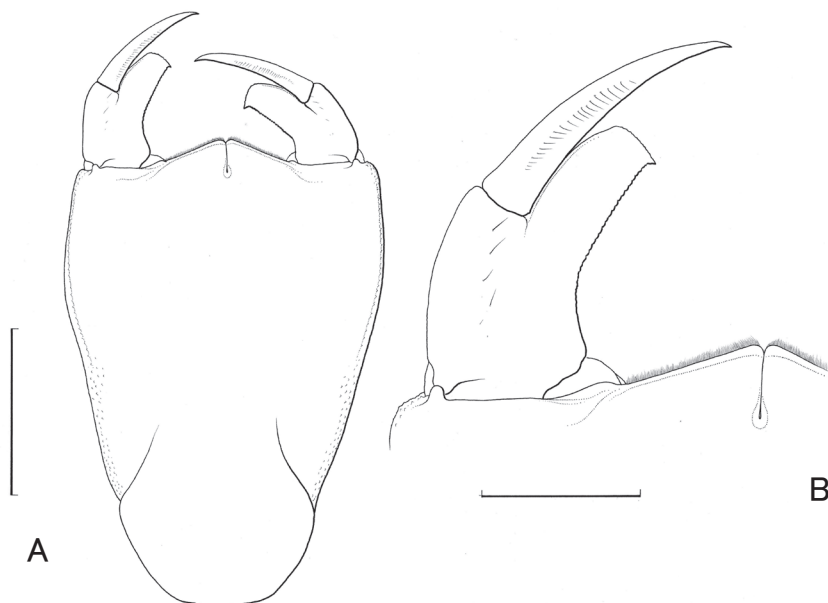


Fig. 5. Larval morphology (Final instar) of *Anax ephippiger*. A. Labium; B. Anterior part of labium, showing left lateral lobe and anterior margin of prementum. All dorsal (inner) views. Scale: 2.5 mm (A); 1 mm (B). Del. KAWASHIMA Itsuro 2021 ©

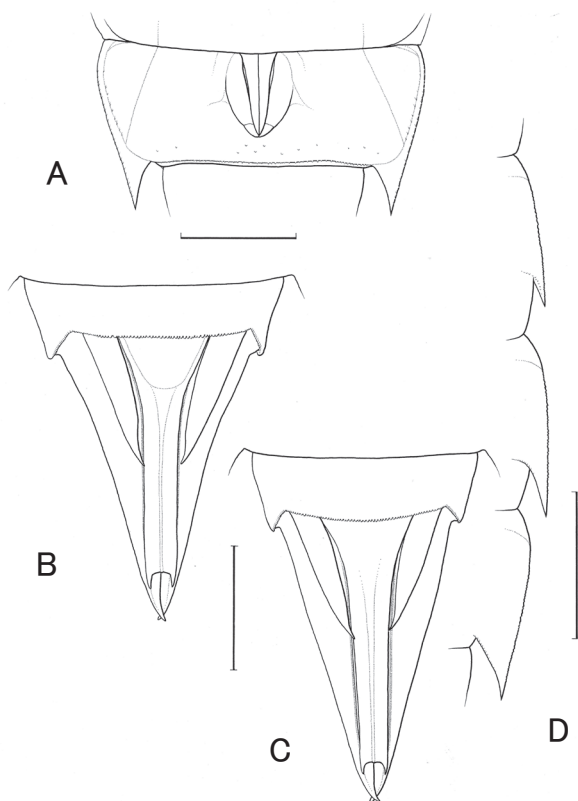


Fig. 6. Larval morphology (final instar) of *Anax ephippiger*. A. 9 & 10th abdominal segments, showing ovipositor on 8th in female; B. Periproct in male; C. Ditto in female; D. Lateral spines of 7th to 9th abdominal segment, right side. All scale: 2.5 mm. Ventral view (A); dorsal views (B, C & D). Del. KAWASHIMA Itsuro 2021 ©

and short, exceeding coxae of mesolegs, but not reaching that of metaleg, in folded situation; prementum 0.63 times as wide as maximum head width; the ratio of longitudinal length to broadest width length 1.41; anterior margin of prementum shallowly produced anteriorly, with numerous densely packed brush-like hairs; median cleft rather short and closed, only a little opened at entrance; no minute projections on sides of the cleft; lateral lobes short, truncated at apex, with an inner hook and many short setae densely colonized on middle part of dorsal (inner) surface; inner margins of lateral lobe straight and serrate; movable hooks rather short, slightly and evenly incurved, then sharply pointed; exterior borders of movable hook nearly 1.3 times as long as exterior margins of lateral lobes.

Thorax (Fig. 3A, B) clearly narrower than head capsule width. Pronotum transverse and spindle-shaped, bulging laterally, with a pair of thin transverse glabrous areas on dorsum. Two processes (Fig. 4C) on prothoracic pleura reduced and not developed, lacking setae on the surface. All wing sheaths parallel, 3 times as long as wide in hind ones; apices of hind ones reaching posterior margin of S4 or anterior part of S5. Legs slender, rather long; distal end of meta-femora reaching S4 in exuviae; tarsal formula 3-3-3; 1st tarsomeres shortest, obliquely articulated to 2nd; 3rd ones longest, a little longer than total length of preceding two tarsomeres in pro- and mesolegs, while almost same length in metalegs. Claws small and simple.

Abdomen (Fig. 3A, B) elongate, subparallel-sided but weakly dilated to the widest on S7, and then, gradually narrowed towards S10 with elongate triangular periproct; each segment transversely both in dorsal and ventral views; lateral spines (Fig. 6D) present on S7 to 9, which is gradually larger distad; dorsal spines absent. Ovipositor sheaths of female (Fig. 6A) small and thick; valvifers not visible from outside; anterior valvulae (Fig. 6A) rather short, the apices of which pointed, almost as long as underlying posterior valvulae, completely covering the latter, not reaching to hind margin of S9 sternite; posterior valvulae almost as long as anterior ones. Coxites (Fig. 6A) rather short and thick, longitudinally spindle-shaped; stylus (Fig. 6A) very short conical and thick, feebly incurved; the apices pointed, 0.18 times as long as length of anterior valvulae; a little exceeding from the apices of anterior valvulae, but not reaching to hind margin of S9 sternite as in anterior valvulae. Periproct (Fig. 6B, C) elongate triangular with sharply pointed; approximately 0.19 in male (Fig. 6B) to 0.21 in female (Fig. 6C) times as long as length of S10; epiproct (Fig. 6B, C) extremely longer than cerci, a little shorter than paraprocts, abruptly narrowed in basal sixth, and then, almost parallel-sided, but very weakly narrowed towards apex; apex shortly bifurcate with a minute spine arising at both sides and with a keel along on mid-line of dorsum; a rudiment of inferior appendage in adult male (Fig. 6B) on dorsal surface of epiproct very shallow, triangular with rounded apex; paraprocts (Fig. 6B, C) sharply pointed, weakly incurved apically; exterior margins feebly incurved or straight with only few minute spines; exterior surface glabrous, without any tubercles and setae. Cerci (Fig. 6B, C) short and thick, with sharply pointed and weakly incurved apex, 0.58 in male and 0.47 in female times as long as epiproct, 0.51 in male and 0.47 in female times as long as paraprocts.

Developmental change of final instar larva (Genta OKUDE & Ryo FUTAHASHI)

GO and RF reared a portion of the eggs laid on November 5, 2019, and 140 first-instar larvae were born between November 18 and 20 (egg duration: 13–15 days). Five of these individuals were photographed daily from the first day of the final instar until adult emergence (Okude *et al.*, 2021). Adults emerged from February 23 to March 10, 2020 (larval period: 97–113 days). Okude *et al.* (2021) revealed that the final instar larvae of Japanese Odonata can be categorized into three stages based on wing morphology: onset of wing expansion (white arrowheads in Fig. 7A) and beginning of wing sheath melanization (black arrowheads in Fig. 7A). The duration of stage 1, 2, or 3 was 9–14 days, 7–8 days, or 3–5 days, respectively. A representative example in each day of the final instar is shown in Fig. 7B, based on the supplementary data of Okude *et al.* (2021).

The compound eye gradually expanded toward the midline during stage 1 (Fig. 7B), as with other Aeshnidae species (Okude *et al.*, 2021). The dorsal compound eye developed during stage 2, and adult-like coloration was observed in stage 3 (Fig. 7B). Thus, it should be noted that the morphology of the compound eye and wing sheaths changes dramatically during the final instar larva.

Discussion

In this paper, changes of morphology, coloration and markings through total instars, especially with the detailed description of the final instar, including the development of the compound eyes and wing sheaths of *A. ephippiger* larva are described. The features above, except for the morphology of the final instar, are illustrated for the first time.

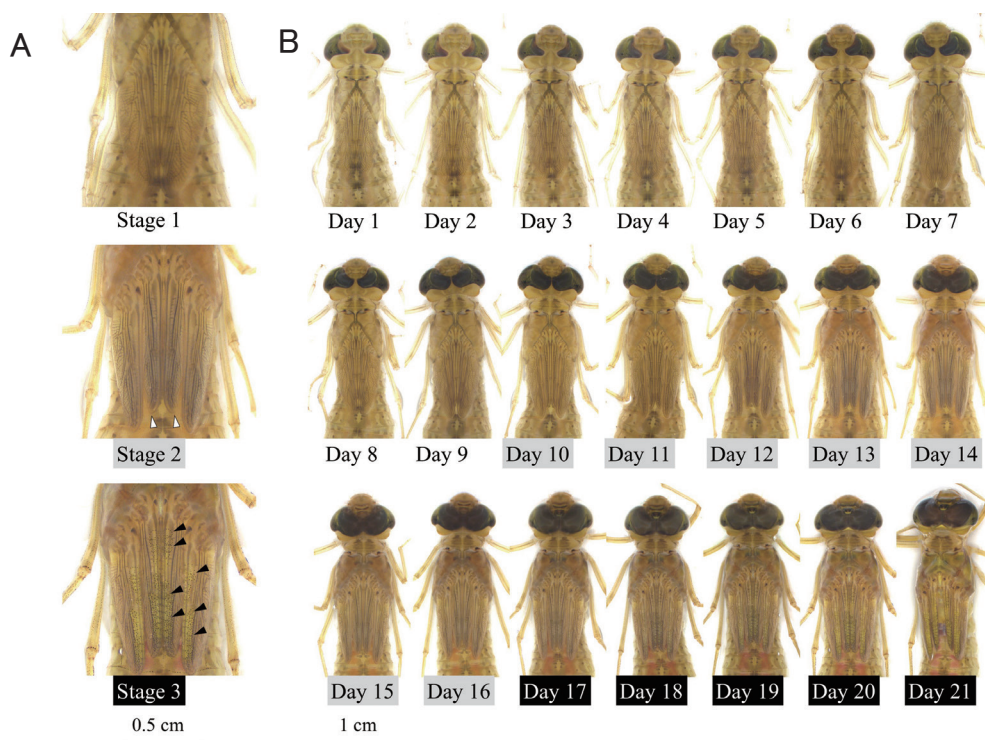


Fig. 7. Last instar larva of *Anax ephippiger*. A. Magnified views of wing sheaths. White arrowheads indicate the dorsal tips of forewings (defining the onset of stage 2), and black arrowheads indicate the black dots and markings on the wing sheaths (defining the onset of stage 3). B. A representative example of daily photos of *Anax ephippiger*. Adult emergence was observed on Day 22. Day numbers shaded in gray or black indicate stage 2 or 3, respectively. Figure modified from Okude *et al.* (2021).

Color change with the larval growth in *A. ephippiger* is generally similar with that in other *Anax* species (Calvert, 1934; Corbet, 1955; Rowe, 1991), which can be categorized into three stages (Fig. 8): at the first stage, dark brown with pale yellowish white maculation; at the second, dark brown with pale yellowish white broad band and marks; at the third, greenish brown with light and shade complicated markings. The first instar larva of *A. ephippiger* is characterized by a rather large pale yellowish pattern, while first instar larvae of other *Anax* are almost blackish brown in color (Fig. 8). The number of instar reaching the third stage, is 7th in *A. ephippiger*, and 7th or 8th in *A. parthenope* Selys, 1839, *A. nigrofasciatus* Oguma, 1915, and *A. panybeus* Hagen, 1867 in YT's experience. In addition, an intermediate color type between the second and third stages was sometimes found in 6th instar (Fig. 2A, B), also as mentioned in *A. papuensis* (Rowe, 1991). In our study, the final instar of *A. ephippiger* is 11th to 13th, whereas that of *A. papuensis* was reported as 14th, if prolarva is not counted as 1st (Rowe, 1991), and in YT's experience, those of *A. parthenope* and *A. nigrofasciatus* are 13th and *A. panybeus* 12th. Therefore, the total number of instars in *A. ephippiger* is almost the same or a little shorter than that of other *Anax* species.

As mentioned in the introduction, the final instar larva of this species has been described several times. Compared with them, there are some differences as follows: the material from Israel by De Marmels (1975) has prementum of labium, anterior margin of which is more roundedly protruded, and, proximal half of prementum is a little narrowed, compared to our materials; in the brief description based on the materials from Ukraine and Nigeria by Butler (1998), the width of lateral lobe of labium seems broader than our materials, and it says "the hinge (of prementum) resting no

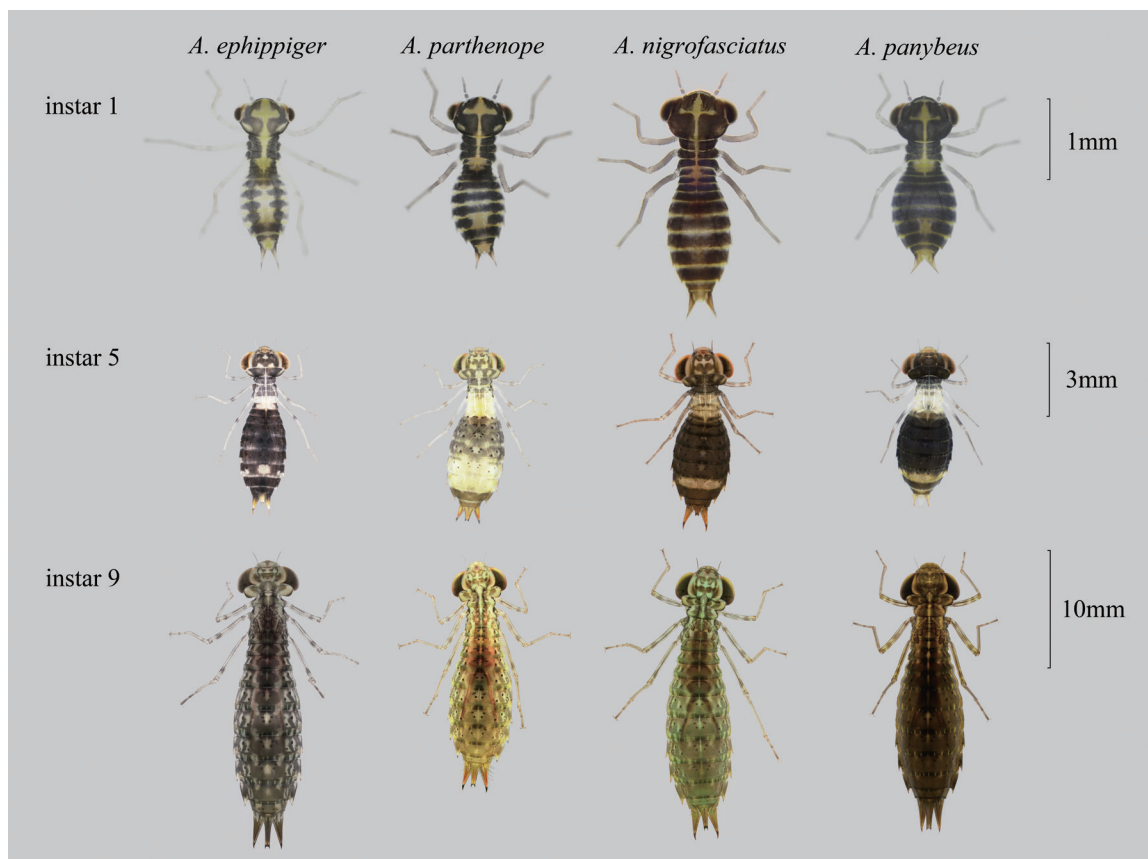


Fig. 8. The comparison of three stages (instar 1, 5, 9 as each representative of 1st, 2nd, 3rd stage) based on color pattern in *Anax* larvae.

further than the 2nd pair of legs”, while, in ours, the latter reaches between mesoleg and metaleg. For the outline of labium and anal appendages, our description is generally consistent with previous studies.

This species has long been considered to belong to the genus *Hemianax*, together with *papuensis*, but many researchers have recently synonymized this genus with *Anax* from the viewpoints of external morphology and paraphyly of *Hemianax* (eg. Peters, 2000; Dijkstra & Kalkman, 2012). In larval character, it has been clearly and repeatedly indicated that *Hemianax* is much near to *Anax*, in rounded head capsule, lateral expended compound eyes, labium morphology, undeveloped prothoracic processes, presence of lateral spines in abdominal S7–9, and short female ovipositor etc. (De Marmels, 1975; Ishida, 1996; Rowe, 1991). Among the known larvae of *Anax* species (Asahina, 1978; Ishida, 1996), the morphology of *A. ephippiger* shares the character that distal side of lateral lobe of labium truncated, not ending incurved hook, with *A. parthenope*, *A. immaculifrons*, and *A. imperator*, however, it is distinct that *A. ephippiger* has relatively short and less narrowed prementum (the length to width ratio 1.4) than the latter three species (the length to width ratio more than 1.7) (Asahina, 1974; Ishida, 1996).

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摘要：笹本 彰彦・古田部 聖・詫間由一・川島逸郎・奥出絃太・二橋 亮：日本産ヒメギンヤンマ幼虫の記載，含：外部形態の変化および発達—日本で得られたヒメギンヤンマの幼虫の外部形態について，初齢から終齢までの形態，色彩・斑紋の変化，終齢幼虫の外部形態の詳細，終齢幼虫の発達の変化を記載した。これらについて，先行研究や同属他種との比較を行った。

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