


Distinct suites of pre- and post-adaptations indicate independent evolutionary pathways of snapping claws in the shrimp family Alpheidae (Decapoda: Caridea)

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Received May 24, 2021

Accepted September 1, 2021

One of the most notable evolutionary innovations of marine invertebrates is the snapping claw of alpheid shrimps (Alpheidae), capable of generating a powerful water jet and a shock wave, used for defense, aggression, excavation, and communication. Evolutionary analysis of this character complex requires the study of a suite of complementary traits to discern pre-adaptations or post-adaptations of snapping behavior. A comprehensive phylogenetic analysis of the Alpheidae based on two mitochondrial and four nuclear markers, covering 107 species from 38 genera (77.6% generic coverage), is presented. Ancestral state reconstruction analyses revealed five independent origins of snapping, two of which relate to the morphologically similar but phylogenetically distant genera *Alpheus* and *Synalpheus*, highlighting significant convergence. The evolution of the five complementary traits (adhesive plaques, tooth-cavity system, dactylar joint type, chela size enlargement, and orbital hood) did not always show a significant correlation with the evolution of snapping overall, sometimes only in a few lineages, suggesting different evolutionary pathways were involved and demonstrating the versatility in the evolution of the snapping mechanisms.

KEY WORDS: Convergent evolution, molecular phylogenetics, parallel evolution, snapping shrimp, systematics.

Key evolutionary innovations have contributed markedly to species diversification in a myriad of groups along with the history of life by facilitating a shift in or expansion of adaptive

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zones (Heard and Hauser 1995; Vermeij 2006; Rabosky 2017). In decapod crustaceans, a number of such innovations have been recognized, including carcinization (Morrison et al. 2002; Tsang et al. 2011), invasions of freshwater, cave, and terrestrial habitats (Ashelby et al. 2012; von Rintelen et al. 2012), and infaunalization (Carmona et al. 2004). Perhaps one of the most notable innovations in decapods is the evolution of snapping claws, which characterize the present second largest family of Caridea,

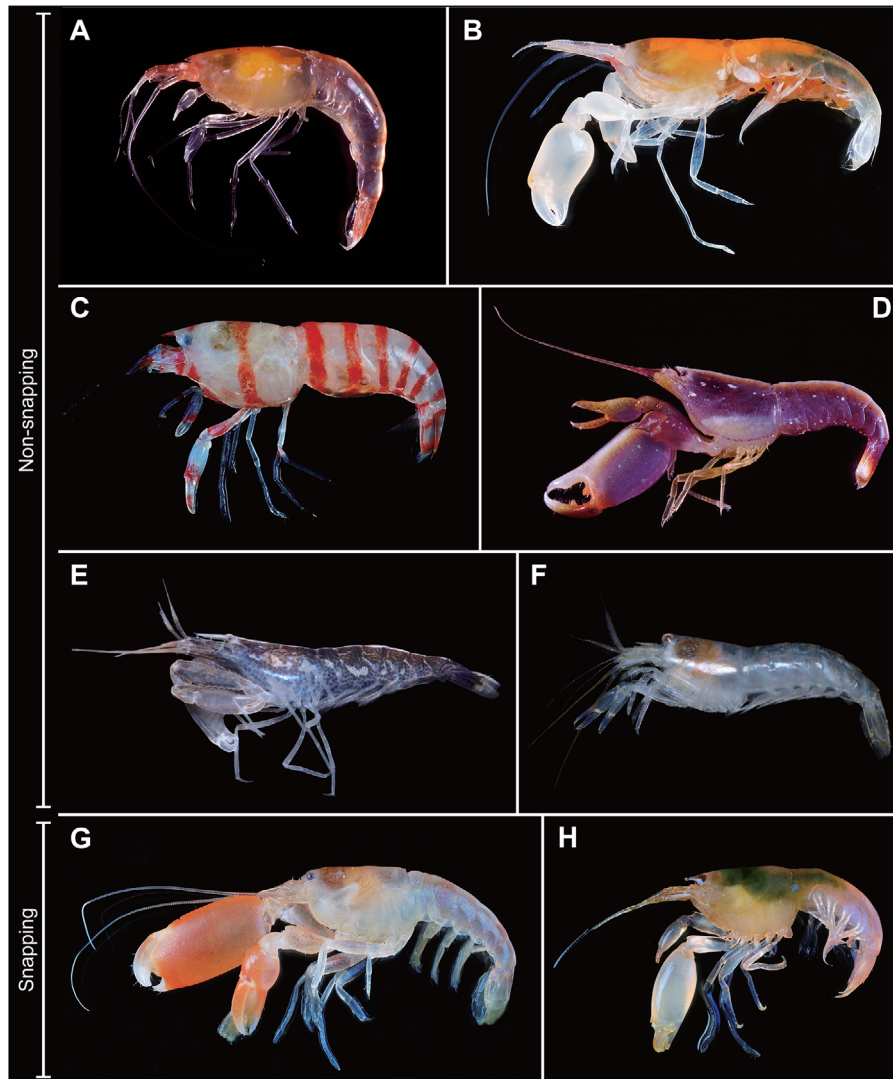


Figure 1. Eight species from different genera of the family Alpheidae, showing the diversity of cheliped size and shape: (A) *Jengalpheops rufus* (Anker and Dworschak 2007), (B) *Automate cf. dolichognatha* (De Man 1888), (C) *Alpheopsis cf. yaldwyni* (Banner and Banner 1973), (D) *Aretopsis amabilis* (De Man 1910), (E) *Athanas japonicus* (Kubo 1936), (F) *Betaeus granulimanus* (Yokoya 1927), (G) *Alpheus barbatus* (Coutière 1897), and (H) *Synalpheus streptodactylus* (Coutière 1905). (A-F) Non-snapping species, (G, H) snapping species. Photographs by Tin-Yam Chan from expeditions organized by the Muséum national d’Histoire naturelle, Paris (A-D, G, H), and Lai Him Chow (E, F).

Alpheidae (Fig. 1), although paralleled by a few Palaemonidae genera (Anker et al. 2006a; Kaji et al. 2018). Among the 750 or so currently recognized alpheid species in 49 genera, snapping claws are present in more than half of the species, most notably in the genera *Alpheus* (>300 species) and *Synalpheus* (>160 species). Their diversification, as well as the emergence of symbioses and eusociality in these two genera (Karplus 1987; Duffy 1996), are likely promoted by several functional significances of the snapping claws, including defense, predation, various intra- and interspecific interactions, rock boring, and burrowing (e.g., MacGinitie 1937; Fischer and Meyer 1985; Schmitz and Herberholz 1998; Atkinson et al. 2003; Tóth and Duffy 2005). Elucidating the evolutionary pathway of snapping claws is,

therefore, crucial to understanding the evolution of alpheid shrimps themselves.

Snapping refers to the extremely rapid claw closure, resulting, at least in some studied taxa (*Alpheus*), in the ejection of a powerful water jet and production of a cavitation bubble, which implodes and generates an audible shock wave (Versluis et al. 2000). The “snap” is used in various intra- and interspecific interactions, as well as communication in eusocial taxa (Tóth and Duffy 2005). The snapping process is controlled by multiple attributes of the chela, including size and applied closer muscle force (Versluis et al. 2000). It is therefore sensible to assume that the snapping mechanism is an evolutionary innovation and represents a character complex involving a set of functionally linked

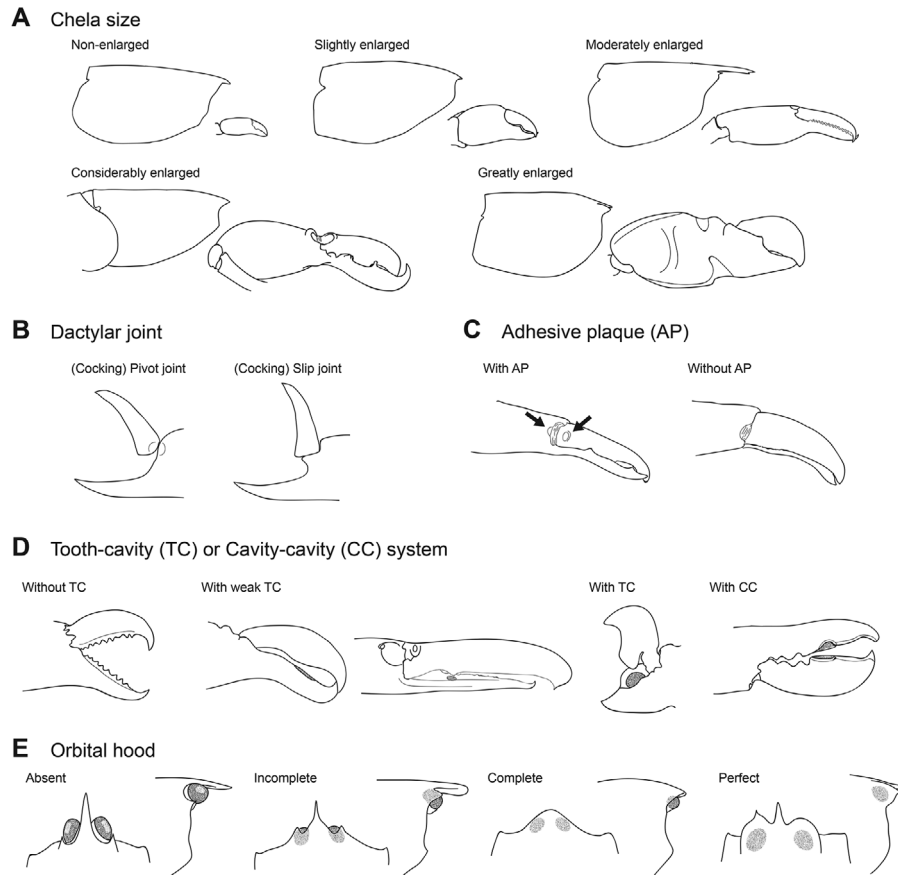


Figure 2. Illustrations of the five complementary traits of snapping: (A) chela size, (B) dactylar joint type, (C) adhesive plaques, (D) tooth-cavity system, and (E) orbital hood. Figures redrawn after various sources.

traits, collectively enabling a wholly new functioning appendage (Anker et al. 2006a). As part of parallel evolution, their adaptive relationship could be defined according to the evolutionary timing: (1) pre-adaptation in the common ancestor, (2) lineage-specific pre-adaptation, and (3) post-adaptation. To obtain a comprehensive picture of the evolution of snapping, studying the evolution of complementary traits in concert is fundamental.

Four putative complementary traits related to snapping are (1) chela enlargement, (2) modification of the dactylar joint, (3) development of adhesive plaques on the chela, and (4) a tooth-cavity system on the cutting edge of the fingers. Chela size in Alpheidae shows huge variation from not enlarged at all to a size wider than the body and reaching half of the body length (Figs. 1 and 2A). Supposing a certain threshold force is required for snapping, snapping taxa are expected to possess a relatively larger chela since chela size is correlated with muscle mass and closing force in other decapods (Levinton and Judge 1993; Claussen et al. 2008). Another trait related to force amplification is the type of dactylar joint (Fig. 2B), in which cocking joints distinctively possess latching and energy-storage mechanisms that allow ultrafast movement and eventually snapping (Kaji et al. 2018). In some

snapping shrimps, cocking is further aided by exoskeletal structures called adhesive plaques, located on the distodorsal palm margin and the opposing dactylar base (Ritzmann 1973; Fig. 2C). During cocking, the two plaques are held tightly by Stefan adhesion, resisting closing of the chela and thus allowing the closer muscle to develop more tension (Ritzmann 1973). Finger armature plays a crucially important role in snapping and indeed many of the snapping taxa exhibit a highly developed tooth-cavity system on the major claw, in the form of a large, plunger-like tooth on the dactylus fitting perfectly into a deep socket on the pollex. In less developed forms, this tooth-cavity system is represented by a small tooth fitting into a shallow depression or a broad bulge fitting into a deep groove; in rarer occasions, both fingers are armed with shallow fossae (Anker et al. 2006a; Bruce 1988; Anker 2019) (Fig. 2D). One additional complementary trait that is not directly related to claw specialization is the development of the so-called orbital hood – an anterior projection of the carapace completely or partially covering the eyes. While the orbital hood is lacking in several “lower” alpeid genera, the remaining groups show varying degrees of its development (Fig. 2E), which is speculated to provide some eye protection against the shrimp’s

own snaps or snaps from intraspecific encounters (Anker et al. 2006a; Coutière 1899).

Despite being a distinctive and ubiquitous group of crustaceans, the intergeneric relationships of Alpheidae remain understudied. The family-level morphological phylogeny of Anker et al. (2006a) remains the only comprehensive analysis performed to date, covering 56 species from all 36 genera known back then. They showed that at least some of the complementary traits were parallel pre-adaptations facilitating the evolution of snapping in alpheids, and discussed the two possible evolutionary scenarios for the evolution of the snapping claw in the family, that is, single versus multiple origins. Previous molecular phylogenetic studies have been restricted to a few species-rich genera *Alpheus* (reviewed in Hurt et al. 2021) and *Synalpheus* (reviewed in Hultgren et al. 2014), as well as the *Betaeus* + *Betaeopsis* clade (Anker and Baeza 2014). A robust molecular phylogenetic framework is, therefore, required to corroborate the results of Anker et al. (2006a), due to high levels of homoplasy resulting in low support for some clades. We performed the first molecular phylogenetic analysis of Alpheidae based on two mitochondrial and four nuclear DNA markers, covering 107 species from 38 genera. We aim to elucidate (1) the origin and evolutionary history of snapping claw and (2) the adaptive relationships between snapping and the five putatively complementary traits.

Materials and Methods

SAMPLING AND DNA EXTRACTION, PCR, AND SEQUENCING

A total of 107 alpheid species from 38 genera were included in this study (Table S1). Total genomic DNA was extracted from ethanol-preserved eggs, pleopods, or pereopods, using the QIAamp DNA Micro Kit (QIAGEN, Hilden, Germany) following the manufacturer's instructions. Partial fragments of two mitochondrial (12S, 16S rRNA) and four nuclear genes (histone 3 (H3), enolase (Enol), phosphoenolpyruvate carboxykinase (PEPCK), and sodium-potassium ATPase α -subunit (NaK)) were amplified using the primers and protocols listed in Table S2. The PCR products were purified using the Millipore Montage PCR₉₆ Cleanup Kit (Merck Millipore, Billerica, MA, USA) according to the manufacturer's instructions, or by the sequencing company (BGI, Shenzhen, China). Sequences were generated using the forward primer on an Applied Biosystems (ABI) 3700 automated sequencer using the ABI Big-dye Ready-Reaction Mix Kit (Life Technologies, Carlsbad, CA, USA), following the standard cycle sequencing protocol.

PHYLOGENETIC ANALYSES

Sequences were aligned using MAFFT (Katoh and Standley 2013) or MUSCLE (Edgar 2004). Alignments of protein-coding

genes (i.e., H3, Enol, PEPCK, NaK) were further confirmed by translating into amino acid sequences to ensure the absence of stop codons. Highly divergent and poorly aligned regions of the 12S and 16S rRNA genes were trimmed using trimAl version 1.3 (Capella-Gutiérrez et al. 2009) with a gap threshold of 20%. The best-fit substitution model for each marker, or each codon position for protein-coding genes was determined using PartitionFinder version 2.1.1 (Lanfear et al. 2017), according to the corrected Akaike information criterion (AICc) (Table S3). The concatenated dataset was analyzed under Maximum Likelihood (ML) with IQ-TREE version 1.6.12 (Nguyen et al. 2015), and under Bayesian inference (BI) with MrBayes version 3.2 (Ronquist et al. 2012). In the ML analysis, branch support was assessed by ultrafast bootstrapping (Minh et al. 2013) with 5000 replicates. In the BI analysis, two independent Markov chain Monte Carlo (MCMC) runs of four chains were performed for 50 million generations, sampling every 50,000th generation. Convergence of chains was determined by having effective sample size (ESS) >200 for all parameters. One-fourth of the trees were discarded as burn-in. All trees were rooted by the outgroup species *Leander plumosus*, *Macrobrachium cf. tenuipes*, and *Palaemon pacificus* (all Palaemonidae).

ANCESTRAL STATE RECONSTRUCTION

Ancestral states of the six traits (i.e., snapping behavior, adhesive plaques, tooth-cavity system, dactylar joint, chela size, and orbital hood) were assessed based on the ML topology with poorly resolved nodes (bootstrap value (BP) <85%) further collapsed using iTOL version 4 (Letunic and Bork 2019) before the analysis.

Coding of the six traits was done on a species basis as listed in Table S4. Specifically for chela size, a proxy for interspecific comparison was calculated as $S = \frac{\sqrt{l \times w}}{cl}$, where l and w represent palm length and width, respectively (major cheliped if unequal), while cl represents carapace length measured from the tip of the rostrum to the posterior margin of the carapace. Length information was retrieved from and averaged over accessible published records and specimens. For polymorphic and sexually dimorphic species, relative chela size was calculated separately for the two chela types or genders, respectively. For specimens of uncertain identity without cheliped information, relative chela size was shown as the range of all congeners, except for *Athanas* that has strong variation within the genus and is not monophyletic in our phylogenetic analysis (see Results). For species without carapace length information, relative chela size was estimated from total length, if available. Given any potential intraspecific variation, interspecific variation in relative carapace length, as well as technical error, the ratios were arbitrarily grouped into five states of enlargement: non-enlarged ($S < 0.15$), slight ($0.15 \leq S < 0.30$), moderate ($0.30 \leq S < 0.45$), considerable

($0.45 \leq S < 0.60$), and great ($S \geq 0.60$). For orbital hood development, we slightly modified the definition by Anker et al. (2006a) and emphasized the degree of eye coverage from the dorsal and lateral sides. Orbital hood was coded as “absent” if eyes are largely exposed; “incomplete” if the eyes are partly concealed dorsally (and laterally); “complete” if the eyes are fully concealed dorsally and partly laterally; and “perfect” if the eyes are fully concealed dorsally and laterally, and in many cases, also frontally.

The ancestral states were reconstructed per trait and for nodes at various taxonomic levels, using a Bayesian approach implemented in BayesTraits version 3.0.1 (Pagel et al. 2004) with the “MultiState” option. State transition was restricted to be stepwise in the analyses of the two continuously varying traits (i.e., chela size and orbital hood) by constraining the rate of non-stepwise transitions as zero. Exploratory reversible-jump MCMC (RJ-MCMC) analyses were first conducted to estimate the boundaries of the priors. Fifty million MCMC generations were run, sampling every 5000th generation, with an exponential hyperprior with the mean drawn from a uniform interval from 0 to 100, and automatic tuning for rate deviation to achieve an acceptance rate of 35%. First one-fourth of the generations were discarded as burn-in. Three independent runs were conducted in the formal analyses with the same parameters applied except with new, constrained priors. Stepping stones sampling (Xie et al. 2010) was performed to assess stationarity among chains via estimation of marginal likelihood (Kass and Raftery 1995) for each chain with 250 stones running for 5000 iterations. Tracer version 1.7 (Rambaut et al. 2018) was used to concatenate the three chains and obtain the mean posterior probabilities (PP) of the ancestral states, and mean and median transition rates. To elucidate the probable coevolutionary pathway of snapping and each of the morphological traits, the ancestral state was re-analyzed for each of the pairs as a compound trait. The transition was restricted to either shifting the state of snapping or the other trait in a stepwise manner. Transitions with 20–60% zero bin (Z) were considered noncritical to the model (Chow et al. 2021).

TESTING OF CORRELATED TRAIT EVOLUTION

Evolutionary covariation between snapping behavior and each of the five morphological traits was tested using the threshold model implemented in the function “threshBayes” of the R package phytools (Revell 2012). Since the analysis only allows binary coding for discrete traits, the correlation between snapping and tooth-cavity system was only tested for taxa with a well-developed system. For the two continuously varying traits, states were converted into numerical pseudo-continuous data with respect to their degree of development, such that it ranged from one (i.e., the least developed state) to K (i.e., the total number of states, also the most developed). In addition, since chela size

data included ranges of values, analyses were run twice with the minimum and maximum values, respectively. The analyses were performed for the entire dataset, as well as trimmed datasets excluding snapping taxa from clade A or S, respectively (see Results), to detect any differentiated correlation signals. Two million MCMC generations were run, sampling every 1000th generation, with a burn-in of 20%. Convergence was assessed by the R package coda (Plummer et al. 2006) based on having ESS > 200 . Additional generations were run if convergence was yet to be reached. Mean correlation coefficients (r) were retrieved and their significances were estimated from the absence of zero (i.e., no correlation) in the 95% highest posterior density (HPD) interval.

Results

PHYLOGENY OF ALPHEIDAE

The phylogenetic trees (Fig. 3; Fig. S1) were constructed based on a concatenated dataset comprising 2850 bp (16S: 544 bp, 12S: 563 bp, H3: 327 bp, Enol: 369 bp, PEPCK: 540 bp, NaK: 507 bp) with a mean missing rate of 4.9% of markers. The ML and BI trees were largely congruent in topology, but the former is better resolved at the deeper nodes; therefore, our inference is mainly based on the ML tree. Seven of the genera were confirmed to be non-monophyletic (*Alpheopsis*, *Alpheus*, *Arete*, *Athanas*, *Automate*, *Leptalpheus*, *Salmoneus*). *Bannereus* was possibly paraphyletic with a divergent specimen of uncertain identity. *Metalpheus* was also potentially paraphyletic, but only supported in the BI analysis. The phylogeny of Alpheidae revealed a basal assemblage and two major clades: A and S, corresponding largely to the “higher alpheids” following the annotation in Anker et al. (2006a) referring to the positions of the two largest genera, *Alpheus* and *Synalpheus*. Detailed results can be found in Supporting Information.

EVOLUTION OF SNAPPING CLAW AND RELATED TRAITS

Our ancestral state reconstruction analyses revealed five independent origins of snapping, originated in the most recent common ancestors (MRCA) of clades A-II, *Nennalpheus* (A-V), *Synalpheus* (clade S-II), and the two lineages of *Salmoneus* (clade S-III), respectively (PP = 1.00) (Fig. 4A). The presence of adhesive plaques was restricted to clades A-II, A-III, A-IV, and A-V, encompassing three of the snapping lineages, with a single-origin traced back to their MRCA (PP = 1.00) and two secondary losses within clade A-III (Fig. 4B). While the gain of snapping and adhesive plaques from the ancestral state proceeded in comparable rate, the latter promoted the former in a hierarchical fashion (Fig. 5A; Fig. S2). Once the derived state was attained, the reversal in either trait was highly limited.

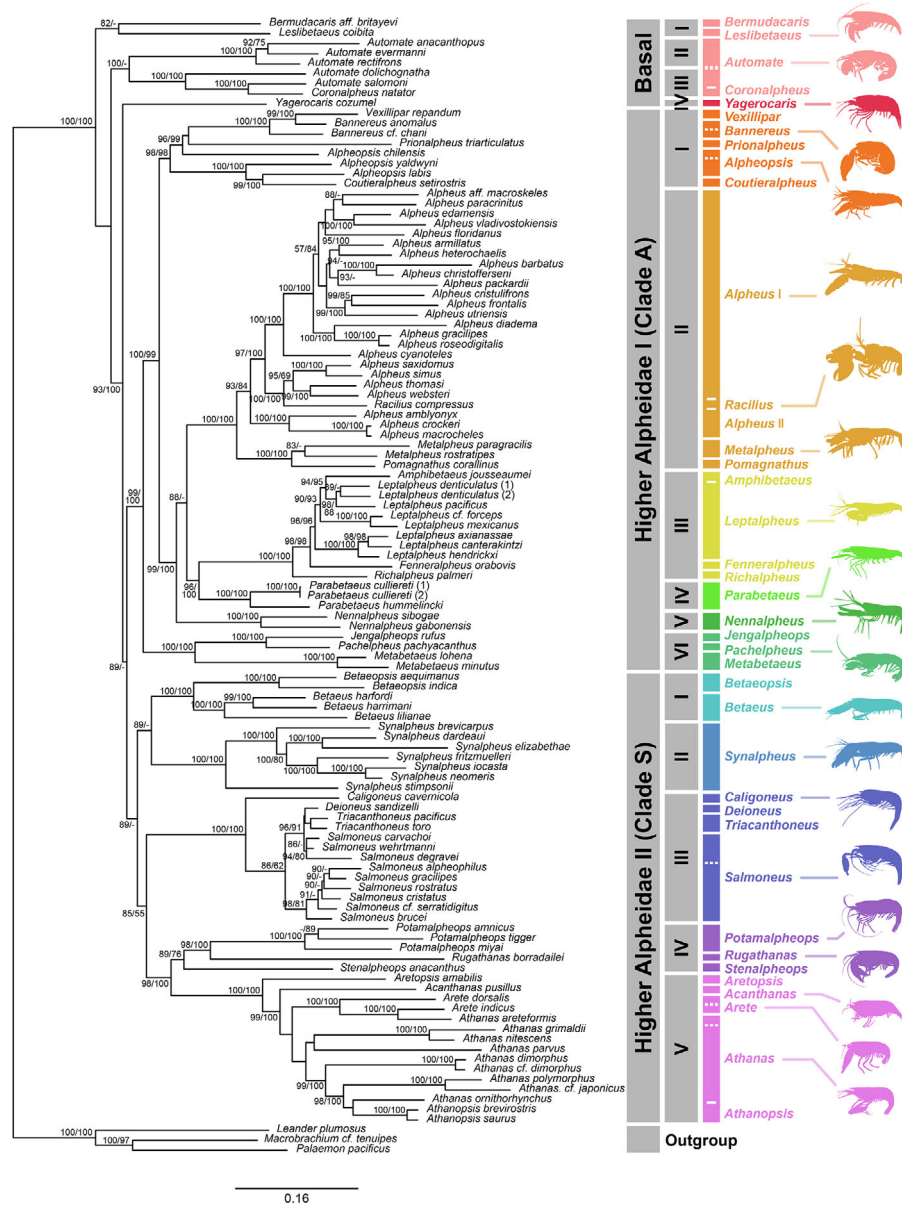


Figure 3. Phylogenetic tree of Alpheidae resolved by maximum likelihood. Branch support values (BP/PP) are indicated as percentages, those with both values <85% are not shown. Major lineages or clades, and genera are highlighted.

Well-developed tooth-cavity systems evolved independently in two of the snapping lineages: clades A-II and S-II (PP = 1.00) (Fig. 4C), with secondary reductions observed in the former clade. Weak tooth-cavity systems evolved three times, all within clade A, one of which involved one of the snapping lineages (clade A-V). The most probable coevolutionary pathway depicted is a gain of snapping behavior followed by a gain of tooth-cavity system, and subsequent shift among variants (Fig. 5B; Fig. S3).

Cocking pivot joint arose in the MRCA of clades A-II, A-III, and A-IV (PP = 0.91), with a reversal to cocking slip joint at the root of clade A-IV (PP = 0.83), suggesting parallel evolution of cocking pivot joints (Fig. 4D). Gaining of snapping behav-

ior and cocking pivot joint from the ancestral state also occurred at comparable rates (Fig. 5C; Fig. S4). Subsequent transition to snapping in the presence of cocking pivot joint was also rapid, with reversal being negligible.

Alpheidae were likely derived from a common ancestor with moderately enlarged chela (PP = 0.88) and incomplete orbital hoods (PP = 0.64) (Fig. 4E,F). Chela size remained more or less similar (i.e., slightly to considerably enlarged) in most of the clades, but with at least four occurrences of size reduction (basal lineage IV, clades A-VI, S-III, S-IV) and five enlargement events (basal lineages I, II, and III, clades A-I and S-V, as well as the snapping lineages of clades A-II and S-II) (Fig. 4E). In

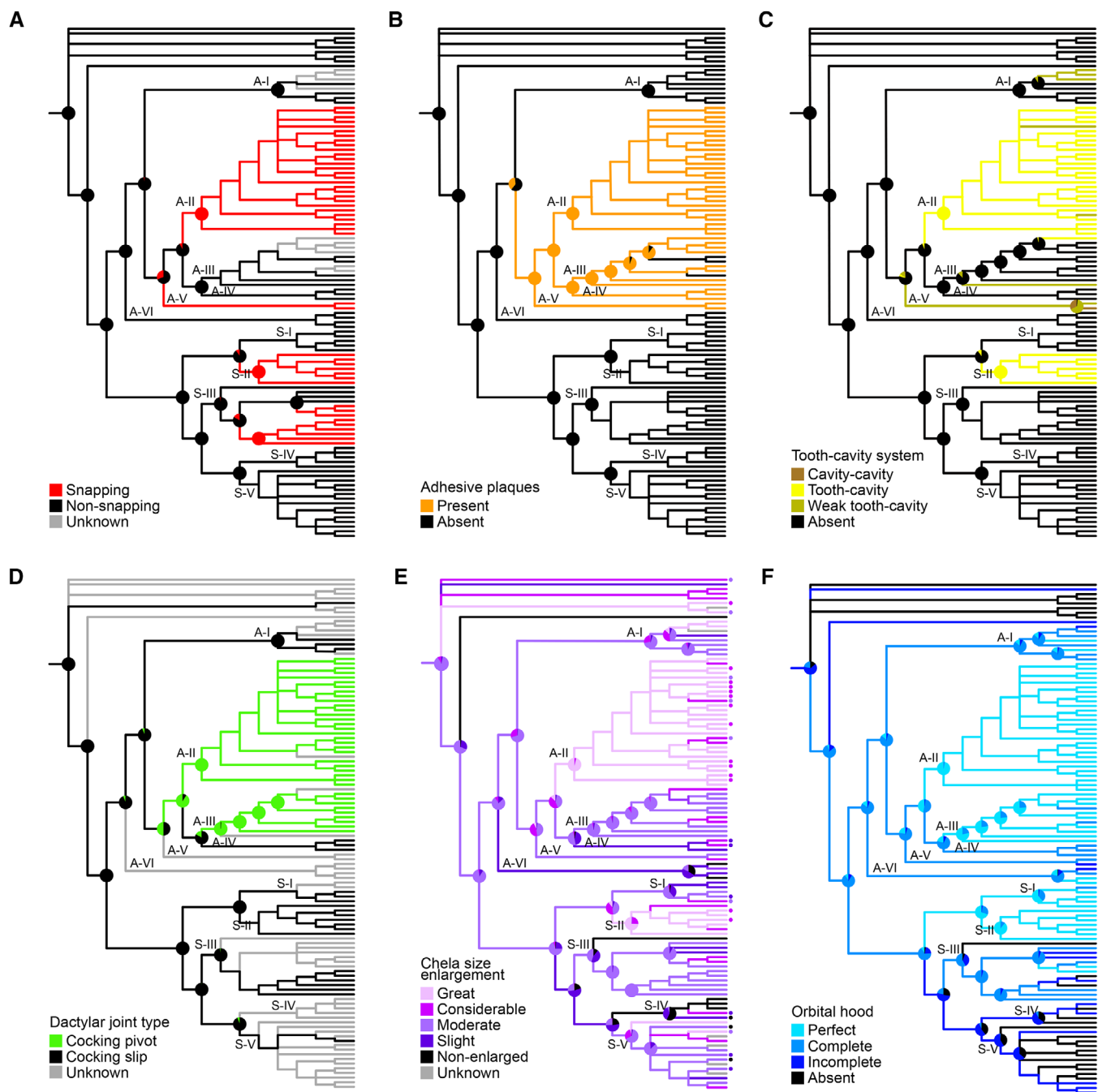


Figure 4. Ancestral state reconstruction of six traits of Alpheidae: (A) snapping, (B) adhesive plaques, (C) tooth-cavity system, (D) dactylar joint type, (E) chela size enlargement, and (F) orbital hood. Branch color represents the most probable state (only the most developed states for chela size are indicated while the least developed states are shown as dots at tips). Posterior probabilities of ancestral states are indicated for selected nodes in the form of pie charts.

contrast, complete orbital hoods evolved early in the MRCA of basal lineage IV and higher Alpheidae (PP = 0.80) (Fig. 4F), and persisted until further independent development in six clades including four of the snapping lineages (clades A-I, A-II, A-III, A-VI, S-I, S-II, S-III) and reduction in clades S-III, S-IV, and S-V. Changes among chela size categories in both the presence or absence of snapping proceeded at comparable rates, except that

transition from “moderate” to “considerable” chela enlargement in snapping taxa was relatively restricted (Fig. 5D; Fig. S5). A similar pattern was observed for orbital hoods, but “perfect” orbital hood represented an evolutionary endpoint for snapping taxa where reduction was limited (Fig. 5E; Fig. S6). Snapping gain likely occurred in taxa with ‘moderate’ or ‘considerable’ chela enlargement, but both transitions were not strongly supported in

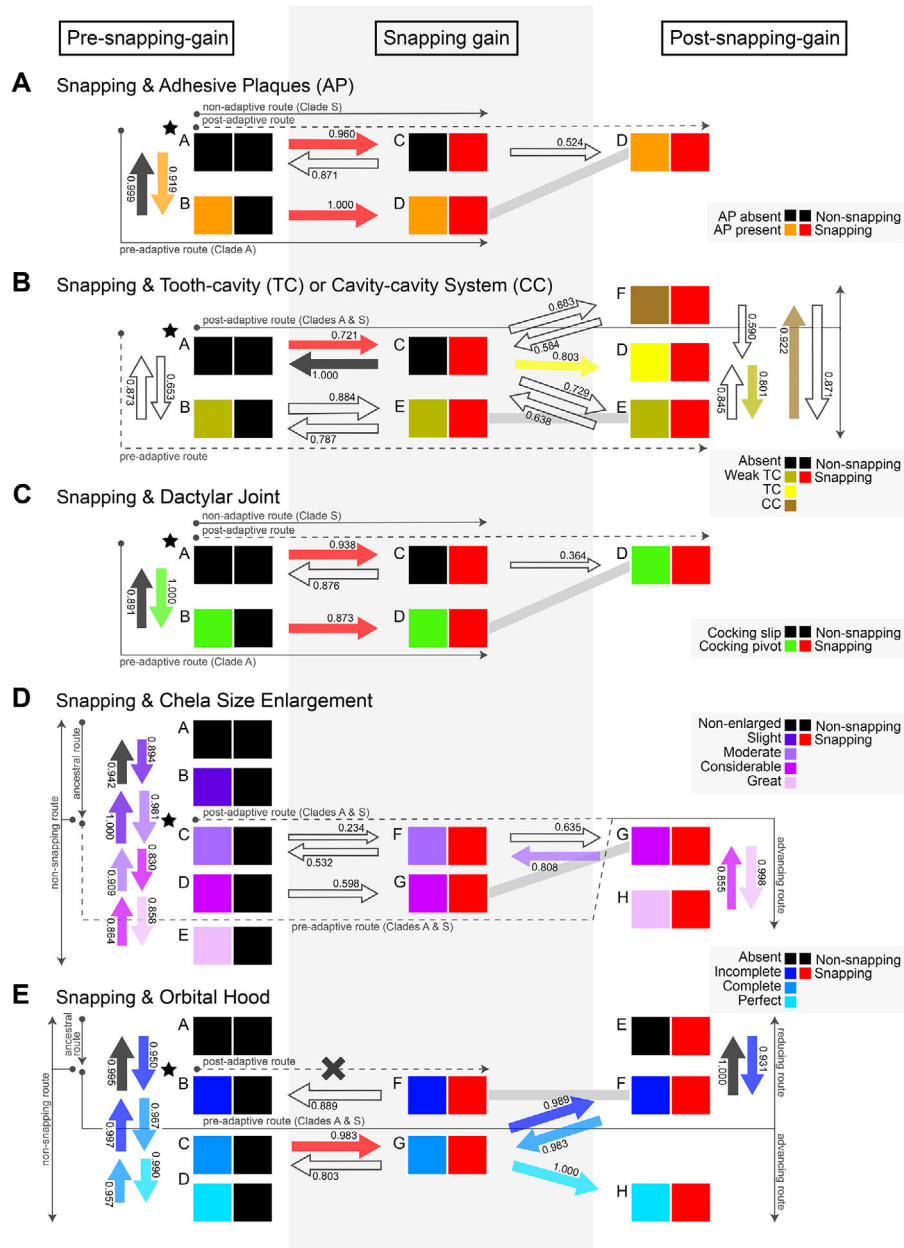


Figure 5. Coevolutionary pathways of snapping and corresponding traits: (A) adhesive plaques, (B) tooth-cavity system, (C) dactylar joint, (D) chela size enlargement, and (E) orbital hood. Stars indicate the ancestral state of Alpheidae revealed by ancestral state reconstruction analyses. Arrows between states represent the direction of transition, with sizes being proportional to the normalized median rate as indicated. Arrow color represents the state being shifted to, except that black and white depict reversals and transitions not critical to the model ($Z = 20\text{--}60\%$), respectively. Transitions with a median rate of zero are not shown. The most probable and less probable evolutionary pathways are illustrated by solid-line and dotted-line arrows, respectively. Crosses on dotted-line arrows indicate further transition is not supported (i.e., zero median rate).

our dataset, probably due to extensive variation within taxon such as sexual dimorphism. On the other hand, snapping gain was only evident for taxa with “complete” orbital hood. While the evolutionary sequence of snapping and chela enlargement from the ancestral state was not clearly elucidated, our results suggest that snapping evolved after orbital hood development, although fur-

ther advances in orbital hoods, as well as chela size, also occurred after the evolution of snapping.

The evolution of a tooth-cavity system, chela size enlargement, and orbital hoods in Alpheidae showed a significant correlation with that of snapping behavior (r ranged from 0.460–0.603) (Table S5). The correlation between the latter

two traits and snapping was, however, not significant when considering snapping taxa of clade A or S only, except between snapping and chela size enlargement in clade S. Adhesive plaques and cocking pivot joint were evolutionarily significantly correlated with snapping only when considering snapping taxa of clade A ($r = 0.483\text{--}0.574$).

Discussion

PHYLOGENY OF ALPHEIDAE

Our molecular phylogeny of Alpheidae is generally in concordance with the previous morphological phylogeny (Anker et al. 2006a) at clade level, but with some significant discrepancies at intra-clade level. The alpeid diversification largely followed a single evolutionary pathway in the morphological phylogeny with the most highly derived and speciose genera concentrated in the crown, whereas our molecular analyses recovered at least two separate evolutionary pathways among the “higher” genera. This suggests that most of the clades are faithfully characterized by morphological synapomorphies, but the presence of homoplasies and autapomorphies, may have caused conflict in the hypotheses at deeper levels.

In the primary taxonomic literature, the genera *Caligoneus*, *Coutieralpheus*, *Jengalpheops*, *Leslibetaeus*, *Potamalpheops*, *Stenalpheops*, and *Yagerocaris* have been considered relatively basal, due to plesiomorphic features such as incomplete orbital hoods, a complete set of coxal mastigobranchs, unspecialized symmetrical chelipeds, and presence of carpal brushes on the chelipeds. Our results, however, showed that only *Leslibetaeus* and *Yagerocaris* are resolved as “basal,” while the others assume relatively “basal” positions among higher alpheids. *Automate*, *Bermudacaris*, and *Coronalpheus* were found to accompany *Leslibetaeus*, whose relatively less derived status has already been hinted in Anker et al. (2006b). The enigmatic *Leslibetaeus*, which is morphologically quite distinct from all other alpeid genera, may represent a lineage that is perhaps closest to the MRCA of Alpheidae, since many of the “basal” taxa in the derived clades superficially resemble *Leslibetaeus* rather than *Automate* and related genera (see below). The cave-dwelling *Yagerocaris*, originally placed in Hippolytidae by Kensley (1988), was found to be a relict lineage without any particular phylogenetic affinity to other genera, supported by its combination of plesiomorphies and autapomorphies (Anker et al. 2006a; Anker 2008).

The evolutionary trend in clade A is hierarchically well-structured. The “basal” genera *Jengalpheops* and *Pachelpheus* (clade A-VI) probably evolved from a *Leslibetaeus*-like ancestor, all showing similar frontal regions, and similarly shaped, small, symmetrical chelipeds. The recovery of *Metabetaeus* in the same clade is intriguing, since it shares little synapomorphies with

other genera but generally agrees with its relatively less derived status among higher alpheids, with a weak affinity to *Alpheopsis* (clade A-I). *Alpheopsis*, *Coutieralpheus*, *Prionalpheus* (clade A-I), *Parabetaeus* (clade A-IV), and *Nennalpheus* (clade A-V) belong to an intermediate group characterized by symmetrical chelipeds, moderately developed rostrum (sometimes reduced), and the sixth pleurite with an articulated plate at the posteroventral angle (Anker et al. 2006a). However, in the present phylogeny, *Bannereus* and *Vexillipar* were found embedded in clade A-I, whereas *Parabetaeus* was recovered as sister to the leptalpheoid generic complex (*Leptalpheus* + *Amphibetaeus* + *Fenneralpheus* + *Richalpheus*) (clade A-III), suggesting that the above grouping is based largely on plesiomorphic features. The derived status of the leptalpheoid complex, as well as *Alpheus* and allied genera (clade A-II), is generally concordant between molecular and morphological analyses. Both clades possess moderately to greatly enlarged, asymmetrical chelipeds, and in particular, a claw folding mechanism and distinctive armature of the fingers in the former clade (e.g., Anker et al. 2006a; Anker 2011), and a well-developed tooth-cavity system in the latter clade (although maybe relatively weakly developed in some taxa).

The evolutionary trend of clade S is less obvious than that of clade A, since there was no apparent “basal” lineage revealed in the present study with incomplete generic coverage. *Betaeus* and *Betaeopsis* (clade S-I) were suggested to be more related to the leptalpheoid genera (clade A-III) based on morphological evidence (Anker et al. 2006a), but were herein recovered in a very distant clade, more precisely as sister to *Synalpheus*. The highly specialized *Synalpheus* is essentially the “counterpart” of *Alpheus* of clade A. Both genera share greatly enlarged chela with prominent tooth-cavity system and well-developed orbital hood, representing convergences in snapping and eye protection-related traits that led to their sister position in previous morphological analysis (Anker et al. 2006a). They, in fact, differ in many other morphological aspects, including details of the snapping claw (Anker et al. 2006a; Coutière 1899; Banner and Banner 1975), reinforcing their separate origins as revealed in our analyses.

Caligoneus (clade S-III), *Stenalpheops*, and *Potamalpheops* (clade S-IV) were considered as morphologically least derived genera (Anker et al. 2006a; Komai and Fujita 2018), but the combination of their “primitive” features may have resulted from secondary reductions and/or reversals. Mirroring the evolution of the leptalpheoid complex (clade A-III), asymmetrical chelipeds with a folding mechanism also evolved in the derived salmonoid (clade S-III) and some members of the athanoid generic complexes (clade S-V), in a parallel evolution. The divergence of *Rugathanas* (clade S-IV) from the athanoid generic complex is surprising given their numerous morphological similarities (including many specific details, see Anker and Jeng 2007) but may be explained by its distinctive cheliped folding mechanism

with the carpus excavated (*versus* merus in the majority of other athanoid taxa) to accommodate the propodus. Nevertheless, a possible affinity between clade S-IV and the athanoid complex has been noted for *Stenalpheops* + *Potamalpheops* and *Pseudathanas*, however, based essentially on the features of the uropodal diaeresis (Miya 1997).

EVOLUTION OF SNAPPING CLAWS IN ALPHEIDAE

Snapping behavior characterizes essentially five alpheid genera, namely *Alpheus*, *Metalpheus*, *Pomagnathus*, *Racilius* (clade A-II), and *Synalpheus* (clade S-II) (Anker et al. 2006a), all with a single, powerful, major snapping claw with a well-developed plunger-fossa snapping mechanism. *Racilius* was confirmed to be nested within the paraphyletic *Alpheus*, while *Metalpheus* + *Pomagnathus* were also potentially embedded within *Alpheus* according to previous morphological and molecular analyses (Anker et al. 2006a; Hurt et al. 2021), but herein recovered as a sister clade to *Alpheus* though. Nevertheless, in all analyses, these four genera belong at least in the same clade and snapping must have evolved in their MRCA. Snapping behavior was also more recently documented in *Nennalpheus* (with a cavity-cavity system on both chelae) and *Salmoneus* (able to produce only weak, barely audible snaps). Our analyses agree on the parallel evolution of snapping (Kaji et al. 2018), although the total number of origins herein recovered was higher, which may be attributed to the uncertainty of snapping in several genera (*Alpheopsis*, *Amphibetaeus*, *Bannereus*, *Leptalpheus*, *Vexillipar*), as well as phylogenetic ambiguity. Should members of these five genera also snap, clade A might share a common snapping origin. On the other hand, snapping might have emerged only once in clade S-III since the hard polytomy might have imposed constraints on the ancestral state. On the basis of the available evidence, snapping likely emerged at least four times in Alpheidae, more specifically, two times each in clade A and clade S, respectively.

Our results suggest that some of the putatively complementary traits show a strong correlation with snapping in Alpheidae overall, whilst the remaining traits only show such correlation in one of the main clades, suggesting that different evolutionary pathways may have been involved. The evolution of snapping + adhesive plaques or dactylar joint type follows a bifurcating pathway, corresponding to clades A and S. The evolution of adhesive plaques and cocking pivot joints favored the subsequent emergence of snapping in clade A, and thus the two characters are potential pre-adaptations, both related to the enhancement of energy storage. The enlargement of adhesive plaques may further be a post-adaptation, which may have facilitated a greater diversification in the crown genus *Alpheus*. Nevertheless, the lack of parallel evolution of adhesive plaques in our dataset indicates such an adaptive relationship is not a requisite for the emer-

gence of snapping. On the other hand, although cocking pivot joint had a single origin in Alpheidae, its precursor role may be reinforced by parallelism in Palaemonidae, especially *Periclimenaeus* (Kaji et al. 2018). However, dactylar joint type may in fact be a complex trait itself, rendering the inference on the adaptive relationship rather coarse-grained. Cocking joints differ from non-cocking ones mainly by the presence of various dactylar retention mechanisms, one of which in cocking pivot joints is a set of two adhesive plaques (Kaji et al. 2018), which explains their largely synchronized evolution in Alpheidae. The second mechanism recognized, the functional subdivision of closer muscle and internal apodemes (Ritzmann 1974), is not only present in some taxa with cocking pivot joints, such as some *Alpheus* (clade A-II) and *Periclimenaeus* (Palaemonidae), but also in some with cocking slip joints, such as some *Salmoneus* (clade S-III) (Kaji et al. 2018). This replicated burst suggests a certain adaptive correlation, but further inference is hindered by the limited information about muscle mechanics across caridean shrimp in general. Despite this common feature, snapping alpheids of clades A and S clearly evolved snapping via two different pathways regarding the cocking system: that of clade A involved the transition to pivot joints with cocking aided by adhesive plaques (and in some cases, also by subdivided closer muscle and internal apodemes), while clade S retained slip joints but with structural changes such as muscle insertion angle to achieve cocking (Kaji et al. 2018).

Multiple evolutionary pathways are also evident in the evolution of the tooth-cavity system as a post-adaptation of snapping, but are not clade-defined as in the evolution of adhesive plaques and dactylar joint type. Our analysis shows that the evolution of snapping in the presence of tooth-cavity system or its variants is less supported than in the absence of such claw armature, despite the fact that they are frequently referred to as the “snapping mechanism.” From empirical observation, they are apparently not required for snapping, as exemplified by some *Salmoneus*, and several palaemonid genera with a dentate cutting edge on the chela. Nevertheless, in several alpheid lineages, including the two most speciose snapping clades (A-II, S-II), a perfect plunger-fossa system evolved repeatedly, suggesting a functional advantage of this structure. The water jet produced during snapping has been attributed to water displacement when the plunger is driven into the fossa (Versluis et al. 2000), but as a post-adaptation, a tooth-cavity system is likely to help guide the water jet trajectory and accelerate water flow, as there is a tapering channel through in front of the cavity when the chela closes (Coutière 1899; Hess et al. 2013). A perfectly developed plunger-fossa system is an extremely powerful weapon in various biotic interactions, and together with additional ecological functions (e.g., boring into hard substrate), may explain the explosive radiation observed in *Alpheus* and *Synalpheus*. The degree of development of tooth-cavity is highly variable in *Alpheus* and, albeit to a much lesser

degree, in *Synalpheus* (Anker et al. 2006a; Banner and Banner 1975, 1982). Therefore, one of the many remaining questions is the presence of evolutionary hierarchy among tooth-cavity systems. Although a direction from weak to well-developed tooth-cavity system is possible, such transition is not well supported by the present results. Weak or imperfect tooth-cavity systems in other snapping lineages may in fact represent cases of convergence.

Regarding chela size and orbital hood, alpheidids are morphologically predisposed to the evolution of snapping. In cocking joints, the closing force of the claw is not simply proportional to muscle mass and claw size, but related to the proportion of closer muscle contributing to energy storage (Kaji et al. 2018). The presence of cocking aids may further liberate any constraints on snapping claw size. This is essentially why it is possible for taxa with relatively small chela to snap. However, our results suggest that “moderately” enlarged chela represents a minimally required size with the reduction not documented after snapping emerged. This degree of enlargement probably was already present since its divergence, though the initial selection forces remain enigmatic. Further chela enlargement did occur in non-snapping lineages (Fig. 1B,D) but apparently did not favor the subsequent evolution of snapping. In contrast, after the emergence of snapping, there is a tendency towards further post-adaptive chela enlargement. This may be attributed to the consistent selection towards stronger snaps since chela size is correlated with water jet velocity and distance (Herberholz and Schmitz 1999). Although the evolutionary trend of chela size, as well as its adaptive relationship with snapping in Palaemonidae remains unknown and is out of scope of this study, it is an unlikely evolutionary coincidence that the only two caridean families with greatly enlarged chelipeds evolved snapping. In contrast to the single evolutionary pathway towards chela enlargement, the evolution of snapping + orbital hood is relatively more flexible in Alpheidae, despite the advancement of orbital hood from “incomplete” to “complete” being consistently a prerequisite. In some of the snapping lineages, snapping gain is followed by the advancement of the orbital hood to “perfect,” concurring with the long hypothesized concerted evolution (Coutière 1899; Anker et al. 2006a). Interestingly, these lineages (i.e., *Alpheus* and *Synalpheus*) also produce the strongest snaps, due to the presence of well-developed tooth-cavity systems and/or “considerably” to “greatly” enlarged chela, as well as adhesive plaques in *Alpheus*, supporting the hypothesis that protection from snaps is one of the main functional significances of orbital hoods (Coutière 1899; Anker et al. 2006a). However, orbital hoods are certainly not a strict prerequisite of snapping, since this structure is unique to Alpheidae, while snapping lineages also evolved within Palaemonidae without the formation of orbital hoods. Nevertheless, this feature may have facilitated the evolution of snapping in alpheid shrimps by

relieving evolutionary constraints from potential injuries associated with intraspecific encounters. This may be supported by the much higher diversity of snapping taxa, the greater number of independent origins of snapping, and the stronger attainable snap (Kaji et al. 2018) in Alpheidae than in Palaemonidae. This leaves a question what drove the orbital hood reduction in Alpheidae under the presence or absence of snapping behavior, in the present phylogenetic hypothesis. Insights may be gained from investigations on the other functions of the orbital hood using, for example, *Betaeus* with well-developed orbital hood as positive models, and the athanoid generic complex with prevalent orbital hood reduction as negative models.

In summary, the ancestral development of orbital hood and chela enlargement set the stage for the evolution of snapping in alpheid shrimps. The emergence of snapping claws represents a convergence in the two main snapping lineages with different mechanisms adopted to cross the energy threshold. Clade A evolved pre-adaptive adhesive plaques and pivot dactylar joint, while clade S had modifications in muscle dynamics. Post-adaptive development of tooth-cavity systems and further chela enlargement subsequently improved snapping performance in both lineages in parallel, allowing more powerful snaps and leading to a significantly greater diversification in *Alpheus* (clade A-II) and *Synalpheus* (clade S-II) compared to other snapping and non-snapping genera. As snaps became stronger, orbital hood advanced as post-adaptation in tandem to provide additional eye protection from forceful chela closure. The independent evolutionary pathways of snapping claws with distinct suites of pre- and post-adaptations demonstrate the versatility in the evolution of this character complex.

AUTHOR CONTRIBUTIONS

L.H.C. designed the study, carried out lab work and data analysis, and drafted the manuscript. S.D.G. and A.A. sourced the specimens and critically revised the manuscript. K.K.Y.P. carried out lab work and data analysis. K.K.M. helped with data analysis. T.Y.C. critically revised the manuscript. K.H.C. and L.M.T. conceived of and coordinated the study.

ACKNOWLEDGMENTS

The authors thank Kwok Chu Cheung, Wai Wah Wong, Terance Wong, and Yaqin Wang (The Chinese University of Hong Kong) for their technical support, and Laure Corbari and Paula Martin-Lefevre (National Museum of Natural History, France (MNHN)) for allowing us to access MNHN materials. This work was supported by grants from the Research Grants Council of Hong Kong SAR (project no. CUHK14102718, to L.M.T. and K.H.C.), the National Natural Science Foundation of China (project no. 41476146, to K.H.C.), and Ministry of Science and Technology, Taiwan and the Center of Excellence for the Oceans, National Taiwan University (to T.Y.C.).

DATA ARCHIVING

DNA sequences are available from GenBank. Datasets are uploaded to Figshare, available at <https://doi.org/10.6084/m9.figshare.15073059.v2>.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Associate Editor: B. Emerson
Handling Editor: A. G. McAdam

Supporting Information

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

Text S1 Detailed results on phylogeny of Alpheidae

Table S1. Details of the specimens and sequences used in the phylogenetic analyses.

Table S2. Primers and PCR protocols used in this study.

Table S3. Best partitioning scheme and best-fit substitution models selected by PartitionFinder.

Table S4. Character states of the six traits (snapping behaviour, relative chela size, adhesive plaques (AP), dactylar joint type, tooth-cavity system (TC), orbital hood (OH)) coded for the species analysed in this study.

Table S5. Mean correlation coefficients (r) and 95% highest posterior density intervals (HPD) between snapping behaviour and the five putative complementary traits, estimated for all snapping taxa or those in clade A or S only.

Figure S1. Bayesian phylogenetic tree of Alpheidae with posterior probabilities indicated as percentages.

Figure S2. Posterior distributions of the transition rate parameters of the compound trait: snapping + adhesive plaques, with mean rate, standard deviation and proportion of zero bin (Z) indicated.

Figure S3. Posterior distributions of the transition rate parameters of the compound trait: snapping + tooth-cavity system, with mean rate, standard deviation and proportion of zero bin (Z) indicated.

Figure S4. Posterior distributions of the transition rate parameters of the compound trait: snapping + dactylar joint, with mean rate, standard deviation and proportion of zero bin (Z) indicated.

Figure S5. Posterior distributions of the transition rate parameters of the compound trait: snapping + chela size enlargement, with mean rate, standard deviation and proportion of zero bin (Z) indicated.

Figure S6. Posterior distributions of the transition rate parameters of the compound trait: snapping + orbital hood, with mean rate, standard deviation and proportion of zero bin (Z) indicated.